REPRODUCTIVE BIOLOGY AND FLORAL VARIATION IN THE ENDANGERED *Braya longii* AND THREATENED *B. fernaldii* (BRASSICACEAE): IMPLICATIONS FOR CONSERVATION MANAGEMENT OF RARE PLANTS

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Reproductive biology and floral variation in the endangered *Braya longii* and threatened *B. fernaldii* (Brassicaceae): Implications for conservation management of rare plants.

By

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Abstract

The closely related, endangered *Braya longii* and threatened *Braya fernaldii* (Brassicaceae) are endemic to the limestone barrens of the Great Northern Peninsula of Newfoundland. This project determines potential breeding systems, levels of population differentiation, potential hybridization between these species and implications for conservation management. Morphological species differences are also summarized to ensure proper identification of new populations. Results indicate field identification using only single morphological characters is insufficient to unambiguously identify species. Both species were found to be primarily autogamous but *B. longii* has a greater potential for outcrossing than *B. fernaldii.* Cleistogamy was found to occur within some populations of *B. fernaldii,* but not in *B. longii.* Evidence of population differentiation in floral morphology was found for both species and potential hybridization was indicated by floral morphology and hand-pollinations. The results of this study provide guidance for potential conservation management tools and must be addressed before reintroduction and population restoration are implemented.

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General Introduction

An underlying goal of plant conservation management is to conserve the genetic variation present in a taxon and minimize the processes that reduce this variation (Holsinger and Gottlieb, 1991). When dealing with small numbers of populations, the effects of low levels of genetic variation must be considered. Small populations may lose genetic variation resulting in reduced offspring fitness, reduced seed production and increased sensitivity to environmental and demographic stochasticity (Ellstrand and Elam, 1993). This results in an increased probability of extinction in the short term, and reduced potential for evolutionary adaptation in the longer term (Soulé and Wilcox, 1980; Ellstrand and Elam, 1993). The decline of genetic variation is thought to reduce the ability of populations to adapt to changing environments and increase their susceptibility to pest and disease pressures (Beardmore, 1983). These factors become important when attempting to manage rare species, specifically in performing Population Viability Analyses (PVAs), defining Minimum Viable Populations (MVP), planning protected areas, as well as tactics for *ex situ* propagation, and species reintroduction.

PVAs provide an assessment of population persistence and consider the effects of threats such as genetic stochasticity (Menges, 2000). From this, PVAs provide an extinction probability, from which a MVP can be determined (Menges, 1991). MVP is related to extinction probability as larger populations can withstand the threats of stochastic events better than smaller ones (Menges, 1991). Therefore defining protected areas will also depend on the amount of habitat needed to maintain genetic variation of a species. If plants are to be maintained *ex situ*, care must be taken to maintain genetic

characteristics involved in the species specialization to its native habitat (Huenneke, 1991). The design of reintroduction efforts would also consider the effects of genetic variation. Careful matching of source populations with transplant sites is essential for successful reintroduction of species (Huenneke, 1991).

The size of a population and its significance to breeding structure, genetics and evolutionary dynamics is another major concern to conservation biologists (Barrett and Kohn, 1991). The ability of some plant species to self-fertilize suggests they may have reduced gene flow and higher levels of population differentiation (Silvertown and Lovett Doust, 1993). A self-fertilizing population usually has lower genetic variability because the same genetic information is passed from generation to generation, forming a population containing genetically similar members. Therefore, from the perspective of species persistence, the number of populations becomes as important as the size of the population. This also makes the definition of a population more difficult as well as assessing "population's size" for rare self-fertilizing plant species. This problem has not been extensively researched to date. In the literature review for this thesis, there were no cases of conservation efforts dealing with primarily autogamous species.

Many plants exhibit a mixed breeding system of both outcrossing and self-fertilization, but the proportion of breeding resulting from self-fertilization has the largest influence on genetic structure (Barrett and Kohn, 1991). In plant populations, reduced gene flow via seeds and pollen can lead to a population substructuring with increasing levels of self-fertilization (Waser, 1993). Evidence for the loss of genetic diversity in small populations has come from studies of breeding systems (Briggs and Walters, 1997)

because breeding biology significantly influences genetic diversity within and among populations (Loveless and Hamrick, 1984). Breeding systems determine the pattern of genetic transmission and affect the organization of genetic variation in a population (Hedrick, 1990). Breeding systems can differ among populations as well as within populations where plants exhibit different levels of self-fertilization and outcrossing (Mitton, 1992). Understanding the reproductive biology of an endangered species, where there are fewer populations to supply genetic information for future generations, is essential to conservation plans (Andersson, 1995). A population's reproductive strategy affects its effective population size, and the distribution of genetic variation and therefore management strategies for conservation (Menges, 1991).

Geographically isolated, small populations are most likely to suffer from depletion of genetic variation (Menges, 1991). Distinct ecotypes represent discontinuous genetic variation, correlated with specific habitats (Huenneke, 1991). Isolation, therefore, leads to reduced gene flow and population differentiation. When attempting to conserve a plant species, an attempt should be made to conserve genetic material from as many populations as possible (Karron, 1991). Therefore, management strategies should quantify genetic differentiation that may arise from isolation. Population differentiation can be quantified by measuring variation in plant morphology such as flower size among populations (Holtsford and Ellstrand, 1992).

Another concern of conservation biologists relating to breeding system is the effects of hybridization on endangered species. There is evidence that hybridization can have both beneficial and harmful consequences for the conservation of biological

diversity (Cade, 1983). Although it can be used as a last resort to preserve the germplasm of a rare taxon (Avise and Nelson, 1989), hybridization can have a substantial negative impact on rare plant species and may lead to the extinction of rare species through demographic and genetic processes (Levin *et al.*, 1996). A small population with a small number of parents will be more seriously affected by hybridization than will a more abundant congener (Levin, 1975). Hybrids may result when geographic and ecological isolation between species is broken down by human disturbance (Wendt *et al.*, 2001). Contact is likely to take place in disturbed habitats, possibly altering competitive relationships and increasing the likelihood of hybridization if the species are closely related (Anderson, 1949). When contact between species results from anthropogenic forces, it is no longer a natural phenomenon, therefore conservation biologists must take action in preventing loss of parental species through hybridization.

Hybrids may compete with the parental species for habitat space or resources and limit the population growth of the rare species. Hybridization represents a potential road to extinction for many rare plant species, and conservation efforts should be directed at preventing hybridization in rare species (Soltis and Gitzendanner, 1999). Management options may include elimination of the invading species and hybrids from the range of the threatened species, transplanting the threatened populations to more isolated locations, and/or improving habitat by minimizing habitat disturbances (Rieseberg and Gerber, 1995). The first step in incorporating hybridization concerns into management plans is to determine if hybridization is occurring. Hybridization may manifest itself in morphologically intermediate forms (Soltis and Gitzendanner, 1999) and numerous

methods have been used to show hybridity, including intermediate morphology (McDade, 1997) and artificial hybridization (Motley and Carr, 1998).

Natural hybridization, as it related to conservation of rare species can also be beneficial. Hybridization involving one species that is rare and another that is more abundant may potentially result in genetic enrichment of the endangered form (Stebbins, 1942). Natural hybridization may lead to increased fitness, the addition of genetic variability that facilitates habitat expansion, and the hybrid population may act as a genetic reservoir for reconstituting the parental genotypes (Anderson, 1949).

Study Species

The endangered *Braya longii* and threatened *Braya fernaldii* are closely related, endemic species. Both contain relatively few populations and these are geographically isolated. It is also thought that both species are self-fertilizing but little is known about the amount of genetic variation within each species. Increased anthropogenic disturbances have increased the likelihood of contact between species (Hermanutz and Parsons, 2002) and little is known about the potential for hybridization between them. The Braya Recovery Team is currently taking actions to aid in the conservation management of these species.

Long's braya, *Braya longii* Fern., and Fernald's braya, *Braya fernaldii* Abbe. are members of the mustard family, Brassicaceae (Cruciferae). In 1997, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) designated *B. longii* as endangered and *B. fernaldii* as threatened. The status of each species was re-affirmed in 2000 (RENEW, 2001). Both species are globally, nationally and provincially rare with G1, N1, and S1 designations for *B. longii* and G1, N2, and S2 designations for *B.*

fernaldii. Long's braya and Fernald's braya are restricted to a narrow strip of limestone barrens along the Strait of Belle Isle Ecoregion of the Great Northern Peninsula of the island of Newfoundland, Canada (51° 18' 00''N – 56° 44' 00''W). *Braya longii* occurs in only four sites while *B. fernaldii* is known from 14 sites, as of 2000 (Hermanutz *et al.*, 2002). Long's braya occurs over a distance of 6 km while Fernald's braya occurs over a distance of 150 km on the northwest coast and tip of the Great Northern Peninsula.

Habitat

The Strait of Belle Isle Ecoregion is characterized by tundra-like vegetation and limestone barrens with cool, windy and rainy climate (Banfield, 1983). Both species are calciphiles, restricted to shallow soils and colonizing areas of natural disturbances caused by frost, wind and soil erosion (Hermanutz *et al.*, 2002). Both *B. longii* and *B. fernaldii* occur in either natural areas of shallow calcareous soils, gravelly limestone pavements and turfy areas between rocks, or in areas with loose limestone gravel, such as quarry sites or old abandoned roadbeds (Meades, 1996 a; b). Naturally disturbed habitats for braya are defined as areas with natural small-scale disturbances such as wind, frost and soil erosion (Noel, 2000). Anthropogenically disturbed areas include large-scale, human produced disturbances such as quarrying and clearing for road development. Although both types of disturbances seem to provide the habitat that is essential to brayas persistence, there are major differences between natural and anthropogenic disturbance regimes that may influence long-term viability of populations (Noel, 2000).

Braya longii and Braya fernaldii: conservation efforts

The present distribution of both species has been influenced by loss of habitat from limestone quarrying, road construction and community development (Meades, 1996 a; b). Survival and reproduction of both species may also be compromised by insect herbivores (*Plutella xylostella* (Lepidoptera) and several *Delia* spp. (Diptera)) as well as an unknown pathogen (Hermanutz and Parsons, 2002). As of August 2000, three sites *of B. fernaldii* are within protected areas. Two sites containing *B. longii* are proposed as ecological reserves for protection.

The main goal of the Braya Recovery Team is to secure the long-term persistence of both species. To achieve this goal there have been six ongoing conservation management strategies including scientific research, population monitoring, critical habitat assessment and protection, *ex situ* conservation, education and stewardship, as well as restoration and species reintroduction (Hermanutz *et al.*, 2002). The results from the scientific research and monitoring will enable the production of a Population Viability Analysis (PVA) for each braya species, which will allow a minimal population size to be determined (Hermanutz *et al.*, 2002).

This study will contribute to the goals of the Braya Recovery Team by providing basic understanding of the breeding biology, distribution of genetic information and hybridization between these species. These concepts will aid in performing PVAs and defining MVPs to ensure long-term persistence of both species. More specifically, by establishing species delineations in floral characters, this study will assist in the identification of individuals and populations for proper assessment of risk for each

species. The breeding biology and hence distribution of genetic diversity within populations can aid in determining which conservation management tools are appropriate. An assessment of population differentiation will determine how much variation exists and therefore must be conserved among populations as well as in *ex situ* seed banks and living collections. Finally, in determining potential hybridization between these species, management tools to deal with the harmful effects of hybridization, as well as selecting sites for reintroductions can be evaluated.

Objectives of this thesis

In this thesis I examine several problems facing rare plants and their impact on the conservation management of the endangered *Braya longii* and threatened *B. fernaldii*.

The objectives of this thesis are 1) to compare species differences with respect to floral characteristics that influence the breeding system; 2) to compare aspects of breeding systems that affect the genetic structure and fitness of both species; 3) to determine the level of morphological and reproductive differentiation among populations of *B. longii* and *B. fernaldii*; 4) to indicate hybridization potential between these congeners; and 5) to indicate how the above objectives impact future recovery plans for *B. longii* and *B. fernaldii*.

These objectives were assessed via two approaches: Firstly, I examine variation in floral morphology of both species using floral character measurements that influence the breeding system (Chapter 1). Secondly, I investigate basic aspects of the reproductive biology of *B. longii* and *B. fernaldii* by performing hand-pollinations and examine potential cleistogamy using bud character analysis (Chapter 2).

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Co-Authorship Statement

All manuscripts in this thesis were co-authored with Luise Hermanutz. In all instances I was the principal contributor to project design and proposal, implementation of the field research component, analysis of the data and manuscript preparation.

Chapter 1. Using reproductive characters to distinguish between the closely related, rare endemics, *Braya longii* and *B. fernaldii*

1.1 Introduction

Hamrick *et al.* (1991) emphasized the importance of understanding the distribution of genetic variation within a species for designing strategies to preserve genetic diversity. Genetic variation is necessary for adaptive change; therefore if a species lacks adequate genetic variation it is at greater risk of extinction (Schaal *et al.*, 1991). For conservation management of rare species, maintenance of genetic variation is essential if populations are to be successfully re-introduced in the wild (Schaal *et al.*, 1991). Any genetic variation found within and among populations of a rare plant species must be preserved in order to assure species persistence.

The most easily obtained assessment of variation is to measure phenotypic (morphological) variation, and such variation is often assumed to indicate genotypic variation, local differentiation or ecotypes (Schaal *et al.*, 1991). Morphological differences that distinguish species are considered to be an extension of the variation found within and among populations of that species (Charlesworth *et al.*, 1982). Therefore, phenotypic variation can be used to provide a basic assessment of population differences. If population differentiation in floral morphology indicates genetic differences, then this variation must be considered in conservation management. Conservation objectives should focus on conserving the variation found among populations within a rare species.

As floral morphology has such complex and significant consequences to reproduction, the pattern and range of floral variation is also critical to the study of a

species reproductive system (Diggle, 1992). Knowledge of reproduction is also crucial for conservation of rare plant taxa, as the breeding system significantly influences the genetic diversity within and among populations (Hamrick and Godt, 1989), and is therefore necessary in designing conservation management plans (Hamrick *et al.*, 1991). Some of the variation found among populations and species in characters such as flower size, style length and pollen:ovule ratio is likely due to genetic differentiation (Holtsford and Ellstrand, 1992). Variation in breeding systems can be mirrored by variation in reproductive characters. Such floral characters include degree of stigma exsertion (Baker, 1959; Hermanutz, 1990), flower size (including petal size) (Rollins, 1963; Fishman, 2000), pollen:ovule ratio (Cruden, 1977; Bosch *et al.*, 1998), and temporal (dichogamous) or spatial (herkogamous) separation of anthers and stigmas (Webb and Lloyd, 1986; Eckhart and Geber, 1999). The shift from outcrossing to autogamy can be accompanied by changes in floral morphology such as a reduction in the size of floral organs (Wyatt, 1988). Flowers of autogamous taxa typically have smaller corollas, reduced herkogamy and a lower pollen:ovule ratio compared to flowers of related xenogamous taxa (Wyatt, 1983).

Hybridization can be detected by measuring floral morphology with hybrids generally showing intermediate morphology compared to the parental taxa (Wilson, 1992: McDade, 1997). Hybridization also has a direct impact on management decisions for rare plants (Rieseberg, 1991), such as for species reintroductions, habitat restoration and the establishment of reserves. If populations are small and there is increased loss and disturbance of habitat due to human activities, the risk of hybridization increases (Anderson, 1948). Therefore, a potential management tool would be to decrease human impact. Hybridization can be viewed as a rapidly acting

genetic threat to endangered species (Wolf *et al.*, 2001), therefore it must be assessed in rare plant species.

Study Species

Braya longii and *B. fernaldii* are very similar to one another and have in the past been treated as varieties of *Braya glabella* (syn: *B. purpurascens*) (Boivin, 1967). Although *B. longii* and *B. fernaldii* are similar to *B. glabella* in appearance, they are readily separable from that species by a number of attributes of the fruits and inflorescence (Harris, 1985). A recent revision of the North American Braya (Harris, 1985) concluded that these species are distinct. Harris (1985) stated that although *B. longii and B. fernaldii* are very closely related and could be treated as two varieties of a single species, they are consistently distinguishable based on morphological characters. Morphology and a single allozyme difference show that both species diverged from *B. glabella* var. *glabella*. *Braya longii* is more similar to *B. glabella* than is *B. fernaldii*.

The numbers of floral parts and their size and shape are traits that often serve to distinguish taxa (Diggle, 1992). Proper delineation of these species is important when considering conservation management option. In order to accurately assess risk for both species, the numbers of individuals and populations of each species in the wild must be known.

Harris (1985) used herbarium specimens for morphological measurements, and species delineation was based on few specimens (19 in total) from only a small proportion of the total known number of populations (in several cases, exact locations of populations used are unknown). His sampling regime, and hence conclusions

relating to species boundaries did not encompass the entire range of variation possible in each species; therefore further measurements need to be taken to encompass the entire range of variation of floral characters. Also, individuals have been found that display character ranges outside those detected by Harris (1985) (pers. obser.).

The breeding systems of *B. longii* and *B. fernaldii* are thought to be autogamous (Harris, 1985). Floral features such as uniform white flower colour, small flower size, and proximity of the anthers to the stigma suggest an autogamous breeding system in both species. In 1985, Harris tested a single population (exact location unknown) of each species and determined from a greenhouse study that both species were predominantly self-fertilizing (Harris, 1985). The near 100% fruit set and seed set he found in the study also supports an autogamous breeding system. However, the amount of differentiation in potential breeding types within and among populations is unknown. There is no information to date relating to the hybridization potential between *B. longii* and *B. fernaldii*, although recently human-disturbed habitats have resulted in direct contact between the species. There is no evidence that their distributions overlapped prior to large anthropogenic disturbances such as limestone quarrying, road development and community development (Meades personal communication, 2001).

Braya longii and *B. fernaldii* are morphologically very similar: both are small (1-10 cm tall and 1-7 cm tall respectively) caespitose perennials with linear-spatulate leaves and scapose racemes of small white flowers. Both have a thick capitate style that is 0.4 -1.0 mm long for *B. longii* and 0.65 -1.0 (1.2) mm long for *B. fernaldii*. Stigmas are generally shorter and stouter in *B. longii* flowers than in those of *B.*

fernaldii (Harris, 1985).

Both species are very similar in appearance and are difficult to distinguish in the field when the plants are not in reproductive condition. The characteristics of the fruits (siliques) are thought to distinguish the species: *Braya longii* have glabrous siliques, while *B. fernaldii* have pubescent siliques (pubescence of the silique ranges from simple, bifurcate to stellate hairs; the density and type of hair varies among populations) (Harris, 1985). In the past, petal size was thought to be a good indicator for species (S. J. Meades, pers. comm.), with *Braya longii* having larger petals (4.0 – 5.0 mm long, 2.0 – 3.0 mm wide) compared to *B. fernaldii* (2.0 – 4.0 mm long, 1.0 – 1.3 mm wide) (Harris, 1985).

Both species begin flowering towards the middle of June and start producing fruit in mid-late July, depending on local environmental conditions (pers. obser.). The fruit matures from mid to late August. There are species-specific differences in both fruit (silique) and seed size, with *B. longii* having larger siliques (4-9 mm long and 1-2 mm wide) and seeds (1-1.5 mm long) than *B. fernaldii* (4-7 mm long and 1-1.5 mm wide and 1-1.3 mm long, respectively) (Meades, 1996a b). In both species, seed number per silique ranges from 10-16 on two parietal placentae. The seeds of *B. longii* are heavier than those of *B. fernaldii* (mean of 5 seeds: BL=1.32 mg $\pm$ 0.02 (SE) vs. BF=0.84 mg $\pm$ 0.03; Hermanutz, 1998).

This paper presents an analysis of variation in selected floral characters in *Braya longii* and *B. fernaldii*. The objectives of this study were (1) to carry out phenetic analysis of floral characters that distinguish the two species, and to compare these results to Harris' (1985) distinguishing characters; (2) to determine new species and population ranges for morphological characters and identify any population

differentiation with respect to floral characters; (3) to examine floral characters associated with breeding systems and indicate potential differences between *B. longii* and *B. fernaldii*; and (4) to identify any morphologically intermediate individuals and assess the possibility of hybridization and its importance to the conservation management of these species.

1.2 Methods

Study Populations.

Fieldwork was conducted during July-August of 2000 and 2001 at 11 braya sites on the Limestone Barrens of the Great Northern Peninsula of Newfoundland (Fig. 1.1). To assess the level of species-, and population-specific floral character differentiation, geographically isolated populations were chosen at the extremes and in the middle of their distribution for *B. fernaldii*, while all known populations of *B. longii* with sufficient numbers (> 35 individuals) were sampled. A total of 15 plants were sampled from each of the 11 populations.

The most southerly population of *B. fernaldii* (Port au Choix) was not included due to the unusually warm spring and summer, resulting in the plants being past the proper flowering stage for sampling. Therefore, Anchor Point west and east were chosen to represent the most southerly populations (Fig. 1.1). Burnt Cape and Cape Norman were chosen as most northerly populations, and Watt's Point was chosen as the central population (Fig. 1.1).

Due to the small size of the *B. longii* Anchor Point population, only 4 of the 5 *B. longii* populations were sampled (Fig. 1.1). *Braya longii* sites included 2 sites at the Sandy Cove crusher population, 2 sites at the Sandy Cove airstrip population, Shoal Cove and Yankee Point (Fig. 1.1).

Scoring Characters .

Flowers from populations of *B. longii* and *B. fernaldii* were collected and stored in FAA (Formalin: acetic acid: 70% ethanol 5:5:90 v/v) and measured within nine months (Kearns and Inouye, 1993). Plants were chosen haphazardly, and three fully opened (mature) flowers were sampled from each of the 10 -15 plants in each study population.

Flowers were dissected under a stereoscope (Olympus SZ 40) using forceps, then pinned in to show detail. The image was captured by a video camera mounted on the stereoscope using the Snappy Video Snapshot (Version 3.0) program. One to three images were taken per flower to adequately measure all floral characters. The UTHSCSA Image Tool (Version 2.0 for Windows) was used to take measurements of all the flowers. Eighteen floral characters were measured on all flowers collected (Table 1.1; Fig. 1.2).

Phenetic analysis.

Floral character variation among braya populations was examined by pooling all populations of both species (i.e. species designation was ignored) using four methods. First, frequency distributions were examined for population means for all characters. Bimodality was assessed visually by the presence or absence of two peaks in the graph. Bimodality of character distribution was considered as evidence for the presence of two distinct species. Second, a correlation coefficients matrix (using Pearson product moment correlation coefficient) was calculated among all characters. Third, multivariate analysis (Principal Component Analysis) was performed and characters presently used to define species (i.e. pubescence and petal length) as well

as additional floral characters were used to define individuals (individual plants belonging to specific populations) in the clusters. Lastly, Cluster Analysis was performed by calculating the Euclidean distance between individuals based on floral morphology.

Character means by species and populations.

Population means for floral characters of the two *Braya* species have not been compared to date. Therefore, population means and ranges for each species were calculated. One-way ANOVAs were used to determine significant differences in characters among populations within species. Species means and ranges of floral characters were also calculated to aid in future identifications of populations into either *B. longii* or *B. fernaldii*. Fully nested ANOVAs were used to identify significant variation among species. Populations were treated as random effects because interest is in the population and not in the individuals. Type III Adjusted Sums of Squares were used as other terms were already in the model, and adjusted sums of squares are the additional sums of squares determined by adding each particular term to the model (Minitab, 2000). The Bonferroni correction method for multiple comparisons (Sokal and Rohlf, 1999) was used to compare variation between species and among populations where p-values less than 0.003 are significant.

Minitab (Version 13.31 for Windows) was used to perform all statistical analysis. The character "pollen:ovule ratio" was not included in any of the multivariate analyses due to missing data which affected the outcome of these types of analyses.

1.3 Results

Character variation for individual means -

Frequency distributions were examined for all characters measured. Only those characters showing bimodality are included with the exception of petal length (Fig. 1.3), which is included because it has been used as a key character in distinguishing species (Harris, 1985).

Only two of the floral characters examined showed bimodal distribution, degree of pubescence of the ovary, and limb width (Fig. 1.3a, and Fig. 1.3b), and therefore best delineate the species. The threshold for petal length (4mm), found by Harris (1985) did not clearly delineate species (Fig. 1.3c). All other characters showed unimodal or skewed frequency distributions.

Character correlations –

Pooling individuals of both species, flowers with longer petals and wider petal limbs tended to have longer stamens, greater herkogamy, higher pollen:ovule ratios and glabrous ovaries. In other words, larger flowers are correlated with characters associated with outcrossing. Since they are both correlated with more glabrous ovaries, they are therefore associated with *B. longii.*

Correlation analysis showed strong associations between several sets of characters (Table 1.2). Characters associated with the petal, sepal, and androecium, as well as degree of pubescence, herkogamy and pollen:ovule ratios were highly correlated with most characters except for a few characters associated with the gynoecium (style length, stigma width and stigma exsertion, which did not correlate with many other characters). Petal length was highly positively correlated with limb width ($r = +0.87$), herkogamy ($r = +0.56$) and stamen length ($r = +0.80$) and

negatively correlated with pubescence (r = -0.64). Herkogamy was also highly negatively correlated with pubescence (r = -0.68), and highly positively correlated with limb width (r = + 0.67). The pollen:ovule ratio was negatively correlated with degree of pubescence (r = -0.53).

Plotting the two key characters together, limb width and degree of pubescence, (Fig.1.4a) revealed that the clustering of individuals corresponded to degree of pubescence, but not to the published threshold for limb width (2 – 3 mm for *B. longii* and 1 – 1.3 mm for *B. fernaldii*) published by Harris (1985) for *B. longii* and *B. fernaldii*. There was a wider range in limb width for both species than previously published. Many individuals with glabrous ovaries had narrower limbs than reported for *B. longii* by Harris. Assuming the clusters (Fig. 1.4a) represent two species, then species with glabrous ovaries (*B. longii*) have a range of limb width of 1.14 – 3 mm and the species with pubescent ovaries (*B. fernaldii*) have a range of limb width of 0.75 – 2 mm. Plots of limb width and herkogamy indicated no obvious clusters (i.e. no obvious species delineation) but instead, a rather continuous distribution (Fig. 1.4b).

Principal Component Analysis -

Principal Component Analysis (PCA) assisted in summarizing the patterns of interrelationships found in the correlation matrix. Since degree of pubescence seems to be the only non-continuous character that delineates species (with the exception of sparse pubescence), it was removed from the analysis. The first principal component accounted for 45% of the variation and the next two accounted for 19% of the variation (Table 1.3). Other components accounted for <7% each. The first

component showed the major patterns of correlations detailed above, with high positive loadings (> 0.35) on limb width, stamen length and petal length and substantial positive loadings (> 0.29 < 0.33) on sepal length and width, anther length and herkogamy. This component represents an index of larger flower size along with larger male organs and greater anther-stigma separation. The second component had large positive loadings (> 0.49) for ovary width and style length, and substantial negative loadings (< - 0.28) for anther width and herkogamy. This component represents an index of larger female organs associated with smaller male (anther) organs and a decrease in stigma-anther separation.

A scatterplot of the first two components identified a clear separation into two distinct clusters by principal component one but not by the second component (Fig. 1.5 a). The third component showed no evidence of separation, and was not included. Those individuals with positive values of the first principal component had morphology most similar to *B. longii* (larger petals, larger limb width, and larger sepal size) while the cluster to the left of the PC1 axis had morphological characters that resembled *B. fernaldii*. Species separation (by clusters) was not absolute, with the presence of many intermediates. To identify species within clusters the key characteristics of degree of pubescence and petal length were coded within the scatterplots (Fig. 1.5 b, c). *Braya longii* usually has glabrous ovaries while *B. fernaldii* usually has pubescent (dense) ovaries, yet intermediates of both species have been found in the field. The scatterplot (Fig. 1.5 b) revealed that the cluster on the right consists mostly of glabrous ovary individuals with a few individuals having sparse pubescence. The cluster on the left consisted mostly of individuals with ovaries having dense pubescence while a few individuals had sparse pubescence as well. The

scatterplot for petal length (Fig. 1.5 c) did not discriminate the two clusters very well. Since Harris distinguished the species using the petal length threshold of 4mm, this value was used again in the PCA. There were many intermediates and very few individuals (even within the *B. longii* cluster) had petal lengths greater than 4.0 mm. All individuals in the left cluster had petals smaller than 4.0 mm but individuals in the cluster on the right seemed to have most individuals with a petal length of less than 4.0 mm. This is consistent with the earlier evidence that *B. long*ii's petal size may have a much larger range than previously published. The scatterplot of limb width (Fig. 1.5 d) indicates its ability to delineate species: most of the individuals in the left cluster belong to a group (species) with a smaller limb width, which is associated with *B. fernaldii.* A few individuals in the right cluster had a limb width of less than 1.5 mm, indicating that the range of limb widths is also wider than previously published for *B. longii.*

Since most of the variation was accounted for in the first component, PC1 scores were plotted as a frequency distribution (Fig. 1.6 a), which showed a bimodal distribution corresponding to the clusters seen in the scatterplot. The histogram was also coded for pubescence (Fig. 1.6 b) and limb width (Fig. 1.6 c - petal length was not coded). There was a bimodal distribution for PC1 and when coded for pubescence - the left part of the distribution consisted mostly of individuals with dense pubescence (*B. fernaldii*), while the right part was mostly made up of individuals with glabrous or sparsely pubescent ovaries (*B. longii*). The same trend was seen for limb width. Individuals of the left part of the distribution had smaller limb widths than on the right part, corresponding to *B. fernaldii* on the left and *B. longii* on the right.

Principal Component Analysis indicates that there are two distinct morphological groups but these are not defined by a single, simple character.

Although Principal Component Analysis resulted in two clusters, there were intermediates (i.e. not clearly separated by PC1); (Fig. 1.5 a; Fig. 1.6 a). To identify these intermediates, individuals were coded by population. To clarify the graph, only populations containing intermediates were coded, while all other individuals were coded for species (Fig. 1.7).

If the clusters defined by PC1 correspond to either *Braya longii* or *B. fernaldii,* then all individuals from *B. longii* populations should occur to the right of the PC1 axis and all individuals from *B. fernaldii* should occur to the left of the PC1 axis. There were, however, four individuals from Shoal Cove (a *B. longii* site) located to the left of PC1 axis (Fig. 1.7). One individual from Sandy Cove (anthropogenically disturbed) and two individuals from Sandy Cove (naturally disturbed) were also outliers for *Braya longii* (Fig. 1.7). Watt's Point individuals should have been located to the left of PC1 axis but as seen in Fig. 1.7, five individuals were located to the extreme right of the left cluster (i.e. away from the left cluster) and one individual was located within the right cluster. Therefore there were intermediates from each species that were morphologically different from the rest of that species.

Cluster analysis was performed to verify that some individuals from *B. longii* have floral characters resembling *B. fernaldii* and vice versa (Fig. 1.8). After the analysis was done, each observation was indicated by population, then indicated by species (based on previous identifications). The cluster analysis resulted in two clusters that were separated from one another (based on distance on the y-axis). Although there is a clear separation of species, one individual of *B. longii* (from Shoal

Cove) was located within the cluster for *B. fernaldii*, and several *B. fernaldii* individuals were located within the *B. longii* cluster. Eight of the nine *B. fernaldii* individuals found within the *B. longii* cluster were from Watt's Point and the other individual was from Anchor Point. These individuals were more like the other species based on all of the floral characters in the matrix. Four individuals were outside of the two species clusters (to the extreme right of the graph). Two individuals were from *B. longii* populations (Yankee Point and Shoal Cove) and two from *B. fernaldii* (Anchor Point and Watt's Point). These individuals were morphologically distinct from the two species.

Character means by species and populations -

Significant differences between species for 12 of the 17 floral characters were measured (Table 1.4). Organs associated mainly with the gynoecium (sepal length, carpel length, stigma width, ovary width, style length, and degree of stigma exsertion) show no difference between species. These are the same characters that show no correlations to other floral characters in the correlation analysis (Table 1.2).

There were significant differences among populations (Table 1.5 a) within *B. longii* for all characters except anther length and width, stamen length, herkogamy, stigma exsertion and degree of pubescence (see Appendix 1). There were fewer significant differences for characters among populations within *B. fernaldii*: sepal length, petal length, limb width, stamen length, style length, degree of pubescence, number of pollen grains per anther and pollen:ovule ratio (Table 1.5 b), than *B. longii*.

In order to detect possible hybrids among the individuals sampled, populations containing intermediate individuals identified by PCA were examined. Shoal Cove

limb width mean was lower than other populations within *B. longii*. The minimum value of the range was also lower compared to other populations, and more within the range of limb width measurements for *B. fernaldii*. This was the case for herkogamy in Shoal Cove as well. Degree of pubescence maximum range was higher in Shoal Cove than for other populations within *B. longii*. Individual plants were examined closely within Shoal Cove. Individuals with higher pubescence did not have a smaller limb width or lower herkogamy (characters associated with *B. fernaldii*). However, those with lower limb widths did have lower herkogamy. Therefore these individuals exhibit a mixture of *B. longii* ranges and *B. fernaldii* character ranges, and may be hybrid individuals.

Within *B. fernaldii*, Watt's Point and Anchor Point had high maximum values for petal length and limb width. Watt's Point had a higher range for maximum values for herkogamy than other populations within *B. fernaldii*. Upon closer examination of individuals within Watt's Point and Anchor Point, individuals with larger petal sizes did not have higher limb widths or higher herkogamy. Therefore it is unlikely these were misidentified individuals (*B. longii* plants located within a *B. fernaldii* population), since they have a unique combination of characters more likely for both species.

1.4 Discussion

Phenetic analysis of floral characters delineated the species *B. longii* and *B. fernaldii* with the key characters limb width and degree of pubescence. Separation of species by petal length, as used in the past, is not reliable as there is great overlap in range between species. New species and population means for 17 floral characters have been proposed. Population differentiation was found for 12 of the 17 characters

measured in *B. longii* and 8 of the 17 characters for *B. fernaldii*. Floral characters associated with the breeding systems indicate that *B. longii* has greater potential for outcrossing than *B. fernaldii*. Individuals with intermediate morphology were discovered using PCA and examined more closely, this potential evidence of hybridization is a concern for future conservation management strategies.

Character variation of individuals within the two *Braya* spp. endemic to the Great Northern Peninsula of Newfoundland fall into two clusters, discriminated not by a single character, but by a combination of petal length, limb width, sepal length, stamen length, anther length and degree of pubescence. Petal length alone is not a reliable indicator of whether a population belongs to one or the other species. This is mainly due to *B. longii* encompassing a much wider ranger of petal lengths than previously published, and highlights the importance of including al populations in conservation efforts. The clusters found using Principal Component Analysis correspond to the two named species, *Braya longii* and *B. fernaldii*. Populations at Shoal Cove (four individuals) and Sandy Cove (three individuals) represented outliers of the *B. longii* cluster and the Watt's point population (six individuals) made up the outliers of the *B. fernaldii* cluster. Therefore it can be concluded that there are two separate species but they cannot be separated based on a single character.

Degree of pubescence and limb width are the simplest, and the most reliable characters for discriminating these species in the field. These characters have less overlap in distributions (with the exception of sparse pubescence, which was found in both clusters) and have significant correlations with most other floral characters (with the exception of gynoecium measurements). Although these characters delineate the species, outliers were found in the PCA. Although there was a significant difference

in herkogamy between the species, it did not clearly separate the clusters. The range of measurements for herkogamy in *B. longii* is large and overlaps that of *B. fernaldii.*

In order to manage rare species, there must be precise species delineation in order for risk to be assessed. Sound conservation management relies on valid estimation of numbers of populations and plants within populations of each species. Thus for identification purposes *Braya longii* includes individuals with glabrous ovaries (or ovaries with sparse, simple hairs) and limb widths greater than 1.5 mm, petals 3-5 mm long and herkogamy of 0.2 -1.14 mm. *Braya fernaldii* includes specimens with a high degree of pubescence (simple, bifurcate or stellate hairs), limb widths less than 1.5 mm, petals 1.4 mm long, and herkogamy range of 0.005 - 0.6 mm. Further refinement of this evaluation awaits an assessment of genetic variation between species. There has been no successful species differentiation or assessment of population variation using genetic markers, but measurement of genetic variation should be considered one of the top priorities for management of the *Braya* species, especially in light of possible hybridization between species.

Some of the morphological variation found among populations and between species may have caused by differences in local environments. In many studies, the genetic basis of variation has been determined in common garden experiments (Schaal *et al.*, 1991). A specific goal of this thesis, however, was to test field identification methods therefore garden plants were not used in this study. It is important to note that during *in situ* pollinations, differences between species were found to be consistent (pers. obser.).

Of the characters associated with the breeding system, *B. longii* had significantly greater numbers of pollen grains per anther, higher pollen:ovule ratios

and a higher degree of herkogamy. Therefore with larger flower size, larger limb width, which forms a "landing platform" for potential pollinators, greater number of pollen grains per anther, a greater pollen:ovule ratio and greater stigma-anther separation, suggest that *B. longii* has a higher outcrossing potential than *B. fernaldii*. Several studies have found similar links between relative degree of outcrossing and floral characters. Wyatt (1984) found in studies of two races of *Arenaria uniflora*, that the outcrossing race had larger flowers larger petals and stamens and higher herkogamy than the selfing race. Holtsford and Ellstrand (1992) and Brunet and Eckert (1998) found that outcrossing rate was highly correlated with anther-stigma separation. Although degree of stigma exsertion has been found to correlate with outcrossing rate in some species (Hermanutz, 1991), it is not correlated with outcrossing in others such as in *Gilia* (Schoen, 1982) and was not correlated in *B. longii* or *B. fernaldii* in this study.

The breeding systems of both species must be determined via a comprehensive study involving hand pollinations (Chapter 2). There is a greater potential for outcrossing in *B. longii* as indicated by their floral morphology but this must be verified directly. Differences in breeding systems between the two species means a different management strategy is required for each species.

Population differentiation in floral characters found within both species also has important implications for conservation. When attempting to conserve a plant species, an attempt should be made to conserve genetic material from as many populations as possible (Karron, 1991), in order to ensure this genetic variation is maintained. Therefore a proportion of habitat from all populations of both species must be protected. *Ex situ* strategies must also consider genetic variation among

populations and effort should sample and conserve plants and seeds from all populations. When attempting to reintroduce species into habitats, these population differences should also be considered in order to ensure population persistence.

A number of individuals were phenotypically outside the normal ranges of each species. As previously mentioned, intermediates were found in Sandy Cove and Shoal Cove for *B. longii* and Watt's Point and Anchor Point for *B. fernaldii*. Individual plants at Sandy Cove must be examined closely. Since the outlier individuals were not more similar to *B. fernaldii* based on all characters measured (as indicated by cluster analysis where there were no outliers for Sandy Cove), this population most likely belongs to *B. longii* but contains individuals with greater variation in some characters. A few individuals from Shoal Cove were found to be outliers in both the PCA and the cluster analysis. This is of concern because there is a *B. fernaldii* population located in close proximity to this population and hybridization may be occurring. Only one of the individuals had an overall morphology more similar to *B. fernaldii* as identified by cluster analysis, which could indicate a misidentified individual (although no mixed populations containing both species have been found to date). The *B. longii* population at the Shoal Cove site is anthropogenically disturbed. Therefore seeds from another area might have been introduced unknowingly with the transport of gravel. This population must be examined more closely using appropriate molecular genetic markers, and breeding systems must be determined to find out if these intermediate individuals could be the product of hybridization.

Plants in the Watt's point population are infected with an unidentified virus, and developmental changes in the flowers have been documented in this population.

This may explain the intermediates found in this population. Cluster analysis resulted in most of the individuals within this population being morphologically more similar to *Braya longii*. Watt's point individuals should be re-examined for potential hybrids and/or misidentified plants (i.e. this population may consist of a mixture of *B. longii* and *B. fernaldii*). During fieldwork, a few individuals at Anchor Point were found to have much greater petal lengths and others had less pubescence than previously expected for *B. fernaldii*. A small population (containing a few individuals) of *B. longii* has recently been located in the same area. Prior to July 2000 *B. longii* and *B. fernaldii* had never been found to co-occur. This site is naturally disturbed and the two species do not grow as close to one another as at the Shoal Cove site. Since this population was also an outlier in the cluster analysis it is possible that there are a few individuals of *B. longii* within this *B. fernaldii* site, or there could be greater variation in characters measured at this site. Upon looking at the individuals of Anchor Point more closely, there is no evidence of a "mixed" population containing both species. Instead there are individuals that display certain features in a *B. longii* range and others within a *B. fernaldii* range within the same flower.

There is a concern for potential hybridization at all sites where intermediate individuals were found. Outlier (intermediate) individuals have some character ranges more similar to *B. longii* and other character ranges more similar to *B. fernaldii*. Therefore it is unlikely that these individuals are simply misidentified by species. Breeding systems have not been determined for all populations of each species so potential risk of hybridization (i.e. whether pollen from a different species could actually produce viable seed) is unknown.

Interspecific hybridization occurs commonly in many groups of plants (Soltis and Gitzendanner, 1999) and can have a substantial, negative impact on rare plant species (Levin *et al.*, 1996). Hybridization may lead to the extinction of rare species through demographic and genetic processes (Levin *et al.*, 1996) through inhibiting the ability of plants to reproduce and compete (Soltis and Gitzendanner, 1999).

A small population with a small number of parents will be more seriously affected by hybridization than a larger population (Levin, 1975). Hybridization can be viewed as perhaps the most rapidly acting genetic threat to endangered species (Allendorf *et al*, 2001). Hybrids may also compete with parental species for habitat and resources and as a result limit the population growth of the rare species (Soltis and Gitzendanner, 1999). Also, the increased susceptibility of hybrids to pathogens (Whitham *et al.*, 1994) may result in increased attack on the parental species (Soltis and Gitzendanner, 1999).

Hybridization may pose a risk of extinction for many rare plant species, and conservation efforts should be directed at preventing hybridization between rare species and their relatives (Soltis and Gitzendanner, 1999). Little is known about the consequences of hybridization of two rare species, usually the risk lies between one rare species and a more abundant species (Allendorf *et al.*, 2001). The risks are much greater in this situation where there is potential loss of both rare parental species. Natural contact and hybridization between these species was not a risk before anthropogenic disturbance of habitat. Contact is more likely to take place in disturbed habitats, which may increase the likelihood of hybridization when the species are closely related (Anderson, 1948). Therefore immediate action must be taken to prevent future hybrids from forming. In other words, habitat for re-introductions must

be isolated from other species. A potential management tool for existing hybrids would be to eradicate them as they may compete with the parental species for habitat space or resources and limit the population growth of the rare species (Soltis and Gitzendanner, 1999). Another option would be to transplant the threatened populations to more isolated locations, and/or improving habitat by minimizing habitat disturbances (Rieseberg and Gerber, 1995).

Although outliers for the range of floral morphology characters within Watt's Point, Shoal Cove, Sandy Cove and Anchor Point may not represent hybridization, but perhaps a viral infection, wider range of species variation, or misidentification, the fact that the species now co-occur at two locations means that hybridization must be considered to be a threat to the persistence of *B. long*ii and *B. fernaldii.* The ability of these plants to hybridize must be assessed through hand pollination studies and the intermediate individuals must be identified as hybrids through genetic analysis.

The current study has significance to the conservation management of these rare *Braya* species. Proper identification is crucial to ensure valid conservation ranking and population status. Populations of both species are significantly differentiated and therefore all populations must be represented in *ex situ* collections and protected areas must encompass the full spectrum of populations and capture naturally disturbed habitats. The increased probability of contact between species in anthropogenically disturbed areas may increase the chance of hybridization and this indicates that reintroductions and /or translocations should be done in carefully chosen natural locations where the other species does not reside. Care must be taken in all future conservation efforts and aspects of the plant biology should be considered before management strategies.

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Table 1.1. Description of floral characters measured and a description of each. All measurements were taken to the nearest 0.01 mm.

Character	Description
1 Sepal Length	From base of sepal to tip of sepal (one sepal chosen haphazardly from the 4)
2 Sepal Width	Using same sepal as length measured width of sepal at widest part
3 Petal Length	From base of claw to tip of limb (one petal chosen haphazardly from the 4)
4 Claw Width	Using same petal measured at widest part of claw
5 Limb Width	Using same petal measured at widest part of limb
6 Limb Length	Using same petal measured from tip of limb to tip of claw
7 Stamen Length	Measured from base of filament to tip of anther (one stamen chosen haphazardly from the 4 tall anthers)
8 Anther Length	Using same stamen, measured from base of anther to tip of anther
9 Anther Width	Using same stamen, measured at widest part of anther
10 Carpel Length	Measured from base of ovary to tip of stigma
11 Stigma Width	Measured at widest part of stigma
12 Ovary Width	Measured at widest part of ovary
13 Style Length	Measured from tip of ovary to base of stigma
14 Herkogamy	Tip of stigma – tip of anther (absolute value)
15 Stigma exsertion	Measured as absolute value of carpel length – claw length (petal length – limb length)
16 Claw:Limb ratio	Measured as ratio of claw width: limb width
18 Pollen grains/anther	One undehisced anther was chosen haphazardly and the number of pollen grains was counted
19 Pubescence	Degree of pubescence ranked from 1-4 (1-no hairs (glabrous), 2- sparse, simple hairs, 3-dense bifurcate or stellate hairs, and 4 very dense bifurcate or stellate hairs
20 pollen:ovule ratio	Number of pollen grains per (one) anther / number of ovules in ovary

Table 1.2.Character correlations (Pearson r) among individuals (species pooled). Abbreviations: L=length, W=width, st = stamen, stig=stigma, abs=absolute, herk = herkogamy, exser = stigma exsertion, ratio=claw width: limbW ratio, pg/ant=number of pollen grains per anther, P:O ratio = pollen: ovule ratio. All correlation in bold italics are significant p<0.05.

	sepal L	sepal W	Petal L	limb W	st L	anther L	anther W	stig W	ovary W	ovary L	style L	Abs Herk	ratio	abs exse	pubescence	Pg/anther
sepal W	***0.58***															
Petal L	***0.81***	***0.64***														
limb W	***0.75***	***0.69***	***0.87***													
St L	***0.74***	***0.55***	***0.80***	***0.83***												
anther L	***0.63***	***0.57***	***0.67***	***0.69***	***0.68***											
anther W	***0.42***	***0.40***	***0.5***	***0.5***	***0.43***	***0.78***										
stig W	***0.32***	***0.25***	0.19	***0.28***	***0.35***	***0.26***	0.07									
ovary W	***0.4***	0.18	***0.28***	***0.28***	***0.27***	0.1	-0.07	***0.29***								
ovary L	***0.67***	***0.37***	***0.59***	***0.52***	***0.63***	***0.38***	0.11	***0.36***	***0.60***							
style L	0.04	-0.05	0.13	0.03	0.05	-0.05	-0.01	-0.01	0.12	-0.03						
absherk	***0.46***	***0.46***	***0.56***	***0.67***	***0.75***	***0.61***	***0.49***	0.16	-0.07	0.12	-0.14					
ratio	***-0.48***	***-0.39***	***-0.53***	***-0.68***	***-0.58***	***-0.48***	***-0.33***	-0.15	-0.05	-0.23	-0.06	***-0.50***				
abs exse	-0.14	-0.13	-0.17	-0.24	-0.19	-0.17	-0.19	-0.03	-0.13	-0.07	0.05	-0.18	0.23			
pubescence	***-0.54***	***-0.55***	***-0.64***	***-0.72***	***-0.6***	***-0.68***	***-0.57***	-0.21	0.16	-0.14	-0.05	***-0.68***	***0.57***	0.14		
pg/anther	***0.46***	0.21	***0.41***	***0.41***	***0.45***	***0.37***	***0.27***	0.16	-0.02	***0.34***	-0.07	***0.39***	***-0.38***	-0.09	***-0.39***	
P: O ratio	***0.41***	***0.24***	***0.46***	***0.42***	***0.41***	***0.46***	***0.34***	0.06	-0.19	0.14	0.13	-0.20	***-0.41***	-0.01	***-0.53***	***0.87***

Table 1.3. Character loadings on the first three Principal Components of the analysis of floral characters.

Character	Loading on PC1 (45% of variance)	Loading on PC2 (11.5% of variance)	Loading on PC3 (7.5% of variance)
Sepal length	0.331	0.193	0.065
Sepal width	0.298	0.001	-0.019
Petal length	0.353	0.126	-0.108
Limb width	0.369	0.068	-0.054
Stamen length	0.355	0.075	0.098
Anther length	0.326	-0.185	-0.012
Anther width	0.243	-0.326	-0.231
Stigma width	0.153	0.247	0.588
Ovary width	0.112	0.625	-0.03
Style length	0.006	0.486	-0.381
Herkogamy	0.289	-0.282	0.104
Ratio (claw:limb)	-0.276	0.047	0.102
Stigma exsertion	-0.1	0.057	0.603
Pollen Grains/anther	0.207	-0.154	0.2

Table 1.4. Comparison of species means for floral characters. P-values calculated from nested-ANOVAs. Type III Sum of Squares was used in analysis. Significant values for p<0.003 after Bonferroni correction for multiple comparisons. Sign. = significant, Herk = Herkogamy, Abs. = Absolute, exser = degree of stigma exsertion and CW:LW ratio = claw width: limb width ratio, PG = pollen grains.

Trait	*Species*	*N*	*Mean*	*SE*	*Range*	*Source*	*DF*	*F*	*P*	*Sign.?*
Sepal	BL	79	2.25	0.03	1.78-3.04	Species	1	14.96	0.004	No
length	BF	76	1.84	0.02	1.4-2.4	Pop(Species)	9	11.71	< 0.001	
						Error	144			
Sepal	BL	79	1.11	0.02	0.74-1.57	Species	1	15.84	0.003	Yes
width	BF	76	0.92	0.01	0.71-1.27	Pop(Species)	9	6.92	< 0.001	
						Error	144			
Petal	BL	79	3.81	0.05	2.98-5.12	Species	1	18.09	0.002	Yes
length	BF	76	3.07	0.04	2.35-3.82	Pop(Species)	9	14.78	< 0.001	
						Error	144			
Limb	BL	79	1.75	0.03	1.14-2.93	Species	1	25.98	0.001	Yes
width	BF	76	1.12	0.02	0.78-1.71	Pop(Species)	9	19.35	<0.001	
						Error	144			
Stamen	BL	79	2.79	0.03	1.77-3.63	Species	1	33.3	< 0.001	Yes
length	BF	76	2.24	0.03	1.73-2.85	Pop(Species)	9	6.06	< 0.001	
						Error	144			
Anther	BL	79	0.65	0.01	0.51-0.88	Species	1	57.87	< 0.001	Yes
length	BF	76	0.52	0.01	0.4-0.66	Pop(Species)	9	2.75	0.005	
						Error	144			
Anther	BL	79	0.54	0.01	0.42-0.68	Species	1	22.74	0.001	Yes
width	BF	76	0.46	0.01	0.34-0.59	Pop(Species)	9	3.63	< 0.001	
						Error	144			
Carpel	BL	79	2.21	0.02	1.67-2.67	Species	1	3.11	0.112	No
length	BF	76	2.08	0.03	1.62-2.81	Pop(Species)	9	4.44	< 0.001	
						Error	144			
Stigma	BL	79	0.59	0.01	0.44-0.8	Species	1	4.74	0.057	No
width	BF	76	0.56	0.01	0.4-0.7	Pop(Species)	9	1.91	0.055	
						Error	144			
Ovary	BL	79	0.856	0.01	0.67-1.08	Species	1	0.06	0.813	No
width	BF	76	0.864	0.01	0.66-1.09	Pop(Species)	9	5.79	< 0.001	
						Error	144			
Style	BL	79	0.42	0.01	0.25-0.62	Species	1	0	0.956	No
length	BF	76	0.43	0.01	0.23-0.72	Pop(Species)	9	7.1	< 0.001	
						Error	144			
Abs.	BL	79	0.57	0.02	0.17-1.14	Species	1	84.39	< 0.001	Yes
Herk	BF	76	0.21	0.02	0.01-0.74	Pop(Species)	9	2.02	0.041	
						Error	144			
CW:LW	BL	79	0.33	0.01	0.24-0.49	Species	1	35.61	< 0.001	Yes
ratio	BF	76	0.42	0.01	0.26-0.59	Pop(Species)	9	2.71	0.006	
						Error	144			
Abs.	BL	79	0.19	0.02	0-0.56	Species	1	6.38	0.032	No
exser	BF	76	0.25	0.02	0-0.76	Pop(Species)	9	0.94	0.494	
						Error	144			

Pubes-	BL	79	1.05	0.02	1.0-2.0	Species	1	54.3	< 0.001	Yes
cence	BF	76	2.91	0.08	1.5-4.0	Pop(Species)	9	24.3	< 0.001	
						Error	144			
PG/	BL	79	498.8	18.4	150-976	Species	1	11.87	< 0.001	Yes
Anther	BF	76	269.2	11.7	112-482	Pop(Species)	9	8	< 0.001	
						Error	144			
Pollen-	BL	53	37.95	2.04	11.5-81.3	Species	1	11.69	0.001	Yes
ovule	BF	39	19.91	1.25	10-45.57	Pop(Species)	9	6.63	<0.001	
ratio						Error	81			

Table 1.5 Population means for floral characters for a) *Braya longii*, and b) *B. fernaldii*. P-values calculated from one-way ANOVAs. Only significant results are shown. Significant values for p<0.003 after Bonferroni method correction for multiple comparisons. BL = *B. longii*, BF = *B. fernaldii*, D = anthropogenic disturbance, N = natural disturbance AP = Sandy Cove Airstrip, SC = Sandy Cove Crusher, YP = Yankee Point, Sho Co= Shoal Cove, BC = Burnt Cape, WP = Watt's Point, AncPT = Anchor Point, CN = Cape Norman, and STBN = St. Barbe. * = significant, nd = no data

a)

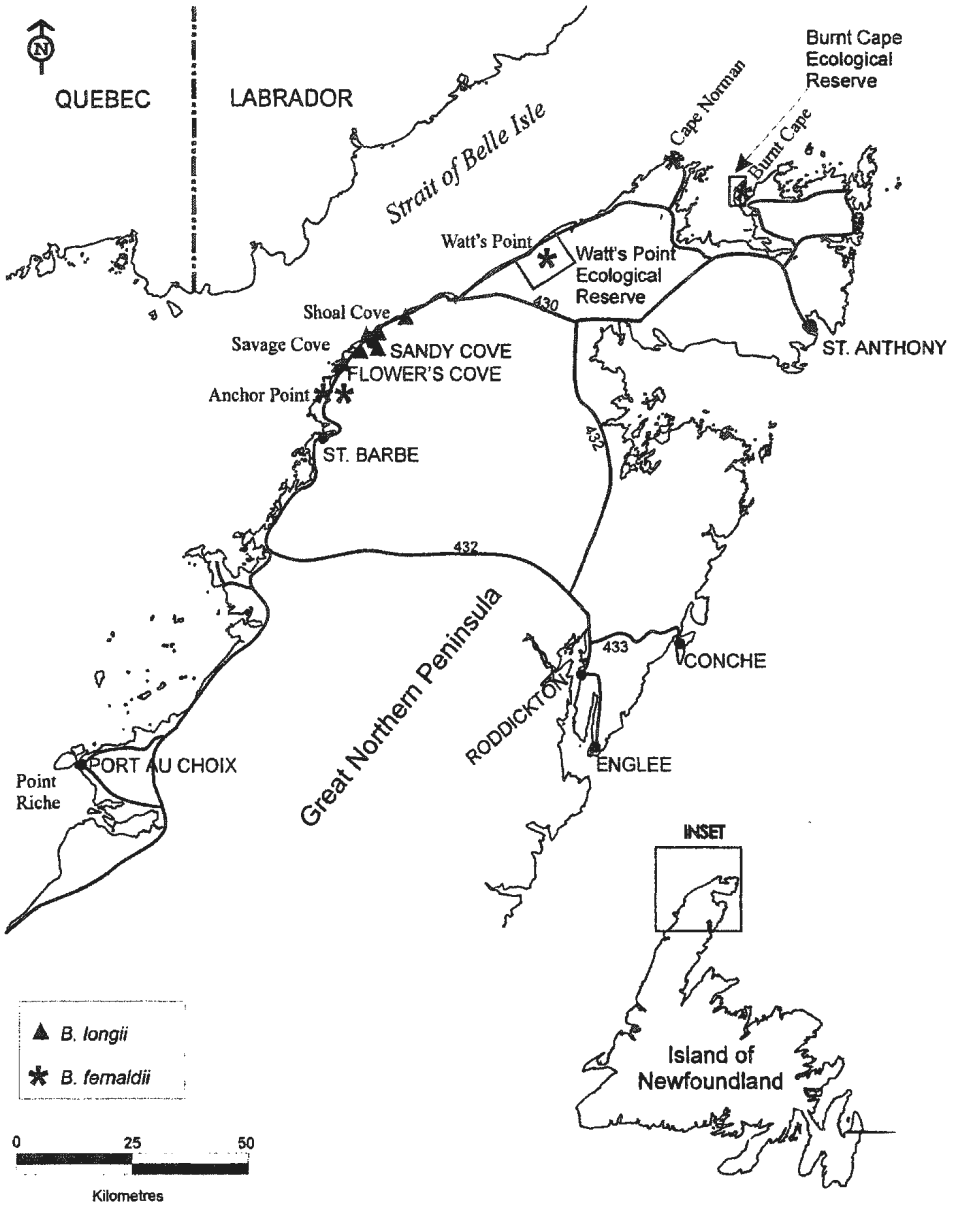
Character	*Species*	*Population*	*N*	*Mean*	*SE*	*Range*	*F*	*p*
Sepal length	BL	APD	16	2.41	0.04	2.00-2.76	15.52	<0.001*
		APN	11	2.55	0.06	2.24-2.97		
		SCD	10	2.13	0.04	1.97-2.37		
		SCN	12	2.03	0.05	1.78-2.28		
		YPD	15	2.33	0.07	1.97-3.04		
		Sho.Co.D	15	2.04	0.03	1.86-2.23		
Sepal width	BL	APD	16	1.02	0.03	0.74-1.16	8.14	<0.001*
		APN	11	1.23	0.04	1.04-1.42		
		SCD	10	1.08	0.03	0.88-1.21		
		SCN	12	1.08	0.02	0.95-1.20		
		YPD	15	1.74	0.04	0.99-1.57		
		Sho.Co.D	15	1.03	0.03	0.77-1.25		
Petal length	BL	APD	16	3.77	0.05	3.36-4.10	12.97	<0.001*
		APN	11	4.49	0.11	3.88-5.02		
		SCD	10	3.69	0.11	3.12-4.23		
		SCN	12	3.56	0.08	2.98-3.91		
		YPD	15	3.83	0.10	3.48-5.12		
		Sho.Co.D	15	3.60	0.07	3.14-4.08		
Limb width	BL	APD	16	1.66	0.04	1.37-1.88	20.43	<0.001*
		APN	11	2.16	0.07	1.63-2.49		
		SCD	10	1.64	0.05	1.38-1.88		
		SCN	12	1.58	0.03	1.33-1.69		
		YPD	15	2.01	0.08	1.68-2.93		
		Sho.Co.D	15	1.50	0.05	1.14-1.73		
Stamen length	BL	APD	16	2.93	0.04	2.56-3.33	5.09	<0.001*
		APN	11	2.89	0.12	1.77-3.25		
		SCD	10	2.67	0.06	2.37-2.92		
		SCN	12	2.72	0.07	2.37-3.18		
		YPD	15	2.85	0.07	2.42-3.69		
		Sho.Co.D	15	2.51	0.07	1.86-2.86		
Carpel length	BL	APD	16	2.32	0.04	2.05-2.57	5.83	<0.001*
		APN	11	2.37	0.06	2.08-2.67		
		SCD	10	2.08	0.06	1.72-2.46		
		SCN	12	2.09	0.05	1.70-2.36		
		YPD	15	2.26	0.05	1.89-2.67		
		ShoCoD	15	2.09	0.05	1.67-2.41		
Ovary width	BL	APD	16	0.86	0.01	0.76-0.97	11.37	<0.001*
		APN	11	0.95	0.02	0.85-1.08		
		SCD	10	0.82	0.02	0.75-0.94		
		SCN	12	0.82	0.01	0.72-0.88		
		YPD	15	0.90	0.02	0.81-1.08		
		ShoCoD	15	0.79	0.01	0.67-0.87		

Style length	BL	APD	16	0.43	0.02	0.31-0.51	8.84	<0.001*
		APN	11	0.50	0.02	0.34-0.62		
		SCD	10	0.41	0.02	0.32-0.49		
		SCN	12	0.46	0.01	0.38-0.54		
		YPD	15	0.35	0.01	0.25-0.42		
		ShoCoD	15	0.43	0.01	0.33-0.51		
Claw width/ Limb width ratio	BL	APD	16	0.31	0.01	0.24-0.34	5.82	<0.001*
		APN	11	0.29	0.01	0.25-0.36		
		SCD	10	0.32	0.01	0.27-0.38		
		SCN	12	0.35	0.01	0.25-0.45		
		YPD	15	0.33	0.01	0.25-0.4		
		Sho CoD	15	0.37	0.01	0.32-0.49		
#Pollen Grains/ anther	BL	APD	14	638.9	56	238-976	5.23	<0.001*
		APN	11	568.8	29.1	368-690		
		SCD	10	439.5	33.2	266-548		
		SCN	12	423.9	22.3	265-549		
		YPD	15	423.3	27.0	150-575		
		ShoCoD	15	490.0	49.5	211-793		
Pollen/ovule ratio	BL	APD	10	49.67	6.43	18.3-81.3	6.26	<0.001*
		APN	10	45.69	3.42	28.3-66.5		
		SCD	10	34.22	2.31	22.8-44.7		
		SCN	10	33.93	2.97	19.9-45.8		
		YPD	10	23.53	2.39	11.5-34.4		
		ShoCoD	3	46.95	7.46	33.6-61.0		

b)

Character	*Species*	*Population*	*N*	*Mean*	*SE*	*Range*	*F*	*p*
Sepal length	BF	BCD	14	1.84	0.03	1.69-2.04	6.43	<0.001*
		WPD	15	2.02	0.05	1.76-2.04		
		AncPt	17	1.76	0.05	1.45-2.20		
		CNN	15	1.90	0.06	1.4-2.28		
		STBN	15	1.72	0.04	1.43-1.98		
Petal length	BF	BCD	14	2.76	0.04	2.38-2.97	17.45	<0.001*
		WPD	15	3.48	0.06	3.11-3.82		
		AncPt	17	3.17	0.06	2.77-3.80		
		CNN	15	2.97	0.09	2.35-3.61		
		STBN	15	2.94	0.06	2.43-3.26		
Limb width	BF	BCD	14	0.98	0.02	1.11-1.44	14.31	<0.001*
		WPD	15	1.26	0.03	1.10-1.98		
		AncPt	17	1.25	0.06	0.93-1.71		
		CNN	15	0.99	0.03	0.78-1.17		
		STBN	15	1.12	0.02	0.99-1.3		
Stamen length	BF	BCD	14	2.24	0.03	2.09-2.5	7.85	<0.001*
		WPD	15	2.39	0.07	1.92-2.85		
		AncPt	17	2.33	0.04	2.08-2.76		
		CNN	15	2.07	0.03	1.73-2.5		
		STBN	15	2.14	0.04	1.94-2.42		
Style length	BF	BCD	14	0.38	0.01	0.31-0.43	5.40	0.001*
		WPD	15	0.40	0.02	0.23-0.72		
		AncPt	17	0.46	0.01	0.38-0.58		
		CNN	15	0.43	0.01	0.30-0.51		
		STBN	15	0.48	0.01	0.40-0.58		

Pubescence		BCD	14	4.00	0.00	4.0-4.0	30.52	<0.001*
		WPD	15	2.82	0.13	1.5-3.33		
		AncPt	17	2.78	0.08	2.0-3.0		
		CNN	15	2.63	0.14	2.0-3.5		
		STBN	15	2.40	0.13	2.0-3.0		
#Pollen Grains/ anther	BF	BCD	10	377.6	20	275-476	8.17	<0.001*
		WPD	9	280.3	19.2	213-385		
		AncPt	12	264.5	27.4	133-482		
		CNN	15	226.9	18.1	112-388		
		STBN	11	222.4	18.1	141-326		
Pollen/ovule ratio	BF	BCD	10	18.86	1.52	12.5-29.8	6.46	0.001*
		WPD	9	17.12	1.43	11.8-23.2		
		AncPt	10	15.86	1.95	10-30.1		
		CNN	nd	-	-	-		
		STBN	8	27.02	3.29	15.4-45.6		



Map revised from National Recovery Plan: *Braya longii* and *Braya fernaldii*

Species	Population	Abbreviation	Disturbance Regime
B. fernaldii	Anchor Point East	Anc. Pt.	Natural
	Anchor Point West	St. Barb.	Natural
	Watt's Point	WPD	Anthropogenic
	Cape Norman	CNN	Natural
	Burnt Cape	BCD	Anthropogenic
B. longii	Yankee Point	YPD	Anthropogenic
	Sandy Cove (Airstrip) 1	APD	Anthropogenic
	Sandy Cove (Airstrip) 2	APN	Natural
	Sandy Cove (Crusher) 1	SCD	Anthropogenic
	Sandy Cove (Crusher)2	SCN	Natural
	Shoal Cove	Sho.Co. D.	Anthropogenic

Fig. 1.1 The Strait of Belle Isle Ecoregion of Newfoundland showing the location of *B. longii* and *B. fernaldii* populations.

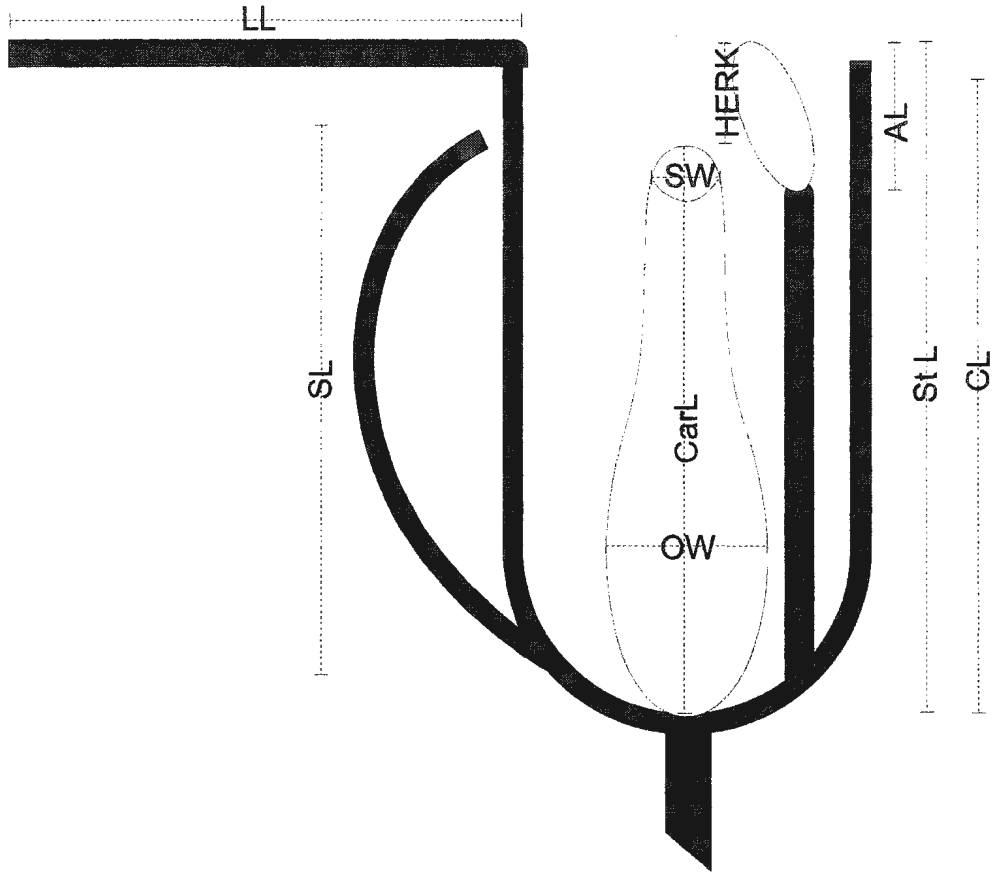


Fig. 1.2 Schematic diagram of a *Braya longii* flower showing most of the traits measured. SL = sepal length, CL = claw length, St L = stamen length, AL = anther length, OW = ovary width, CarL = carpel length, SW = stigma width, Herk = herkogamy, LL = limb length. See Table 1.1 for other measurement descriptions. Figure created by Jolene Sutton, modified from Conner and Via (1993).

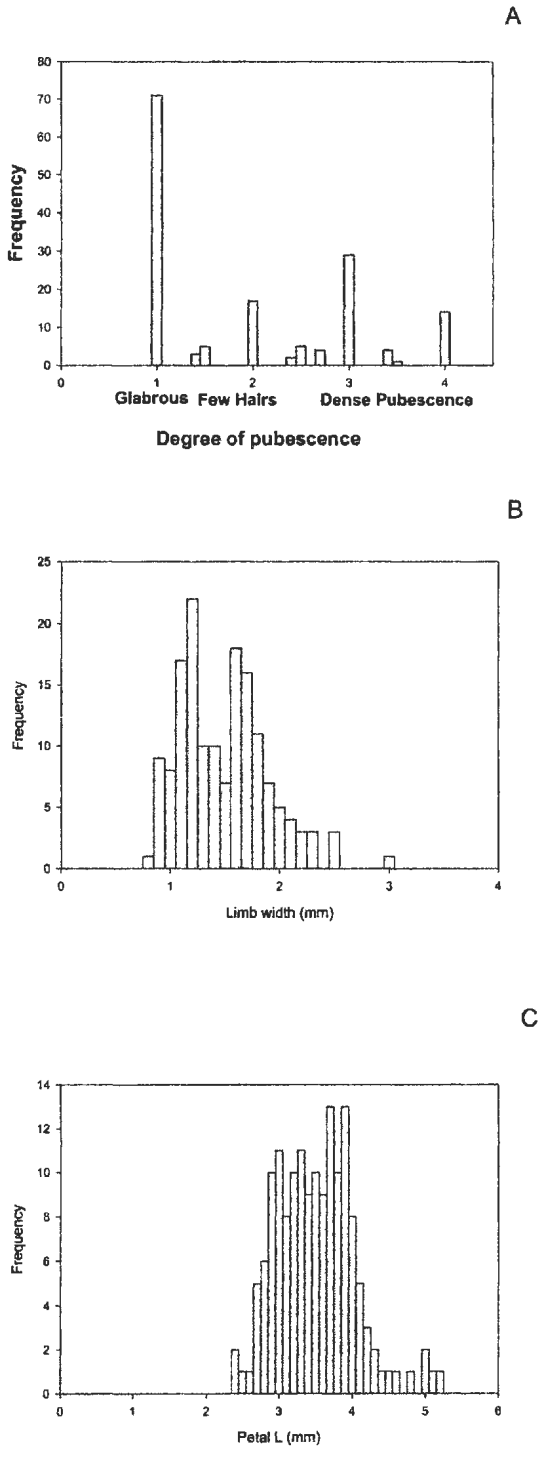


Fig. 1.3. Among population comparisons of floral characters in *Braya* sp. Histograms for a) degree of pubescence, b) limb width and c) petal length

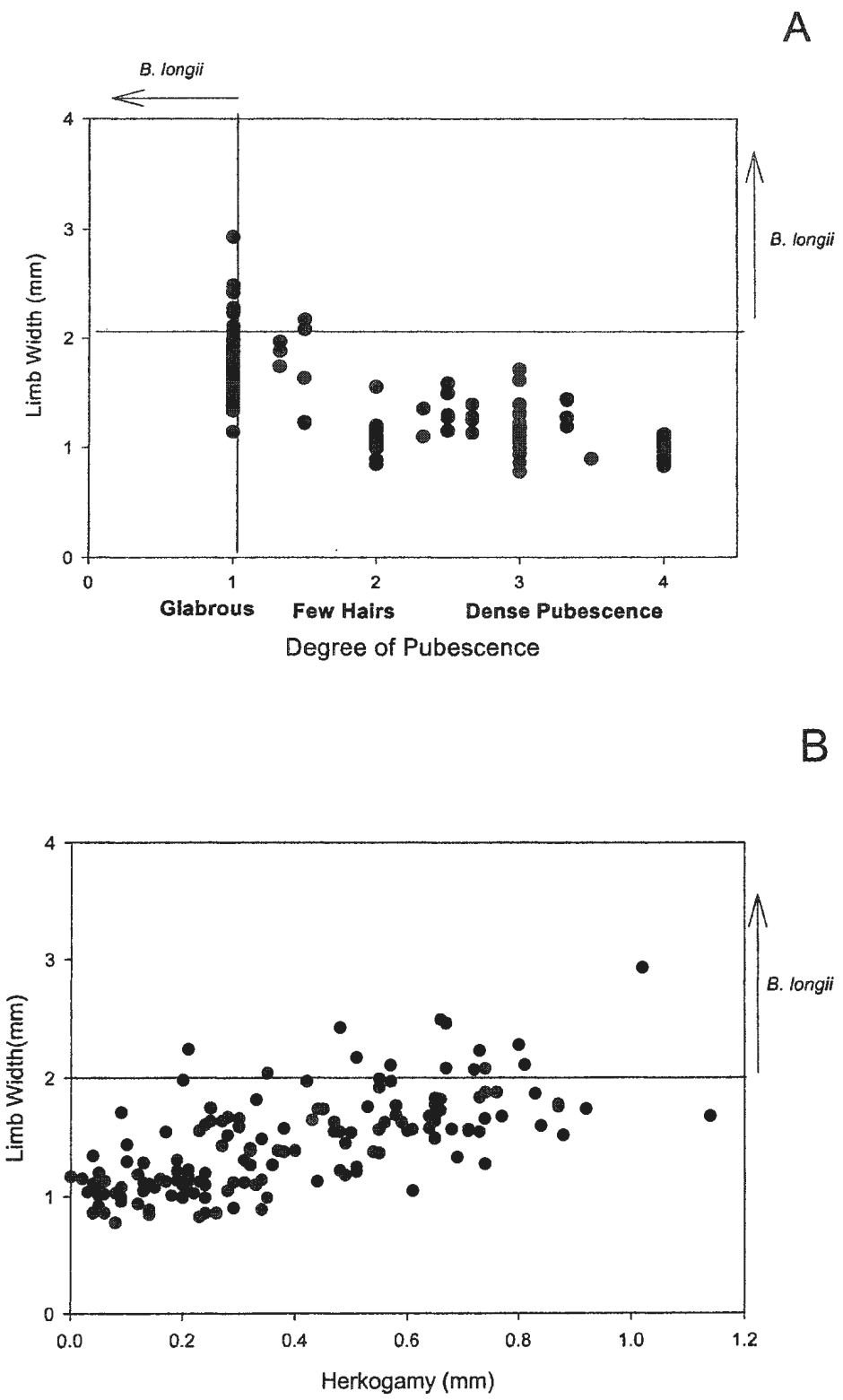


Fig. 1.4. Scatterplot of a) limb width against pubescence and b) limb width against herkogamy for individuals of *B. longii* and *B. fernaldii*. Lines indicate published character thresholds (Harris 1985).

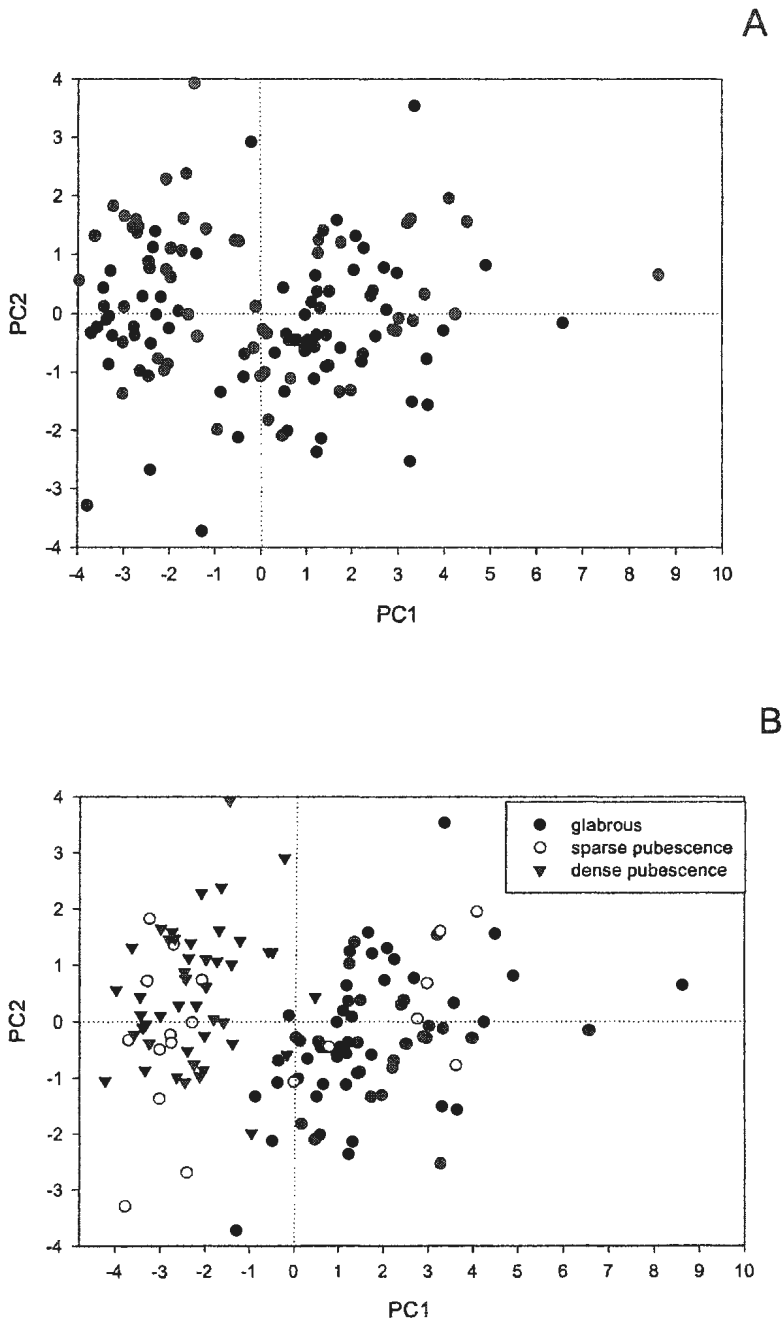
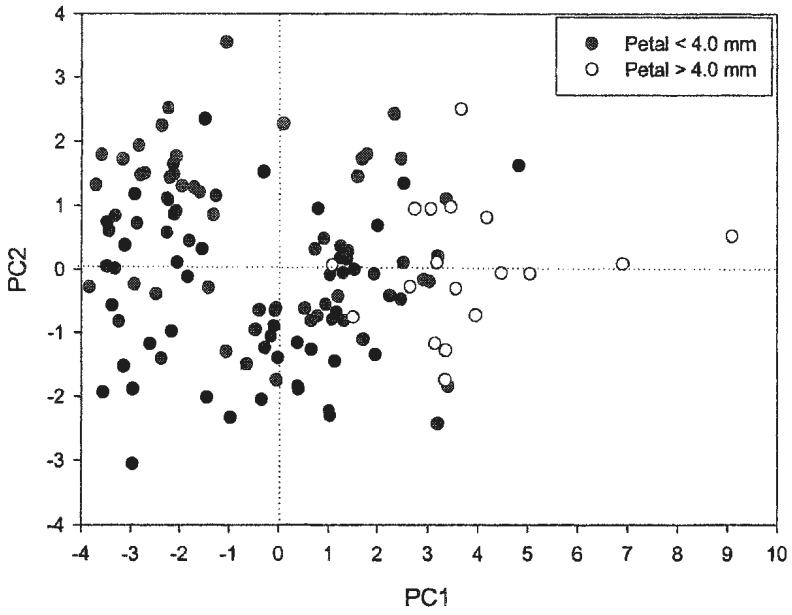
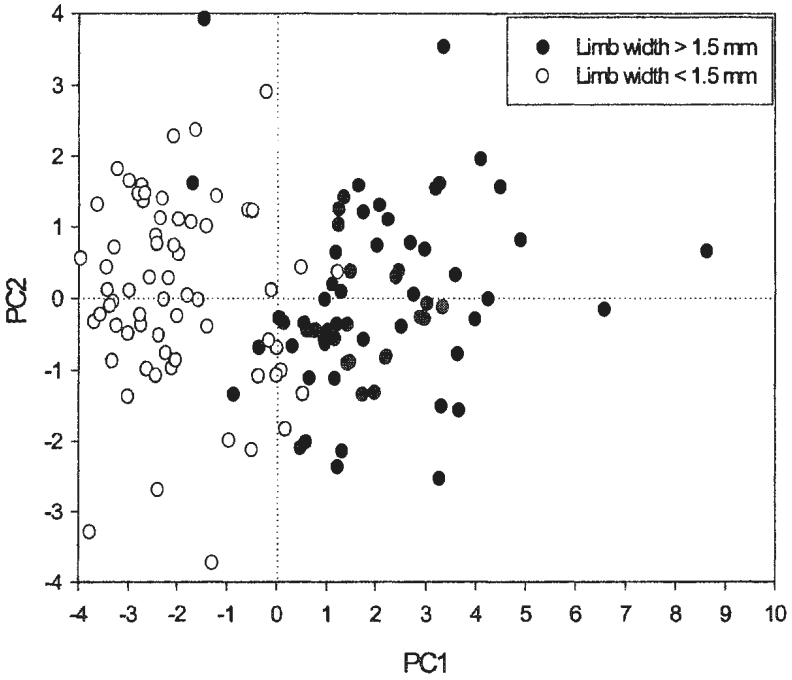


Fig. 1.5. Scatterplots of the first two principal components. Each point represents an individual. (a) Species not coded. (b) coded for degree of pubescence. (c) coded for petal length and (d) coded for limb width.

C


Petal < 4.0 mm
Petal > 4.0 mm
PC2
PC1
4
3
2
1
0
-1
-2
-3
-4
-4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10

D


Limb width > 1.5 mm
Limb width < 1.5 mm
PC2
PC1
4
3
2
1
0
-1
-2
-3
-4
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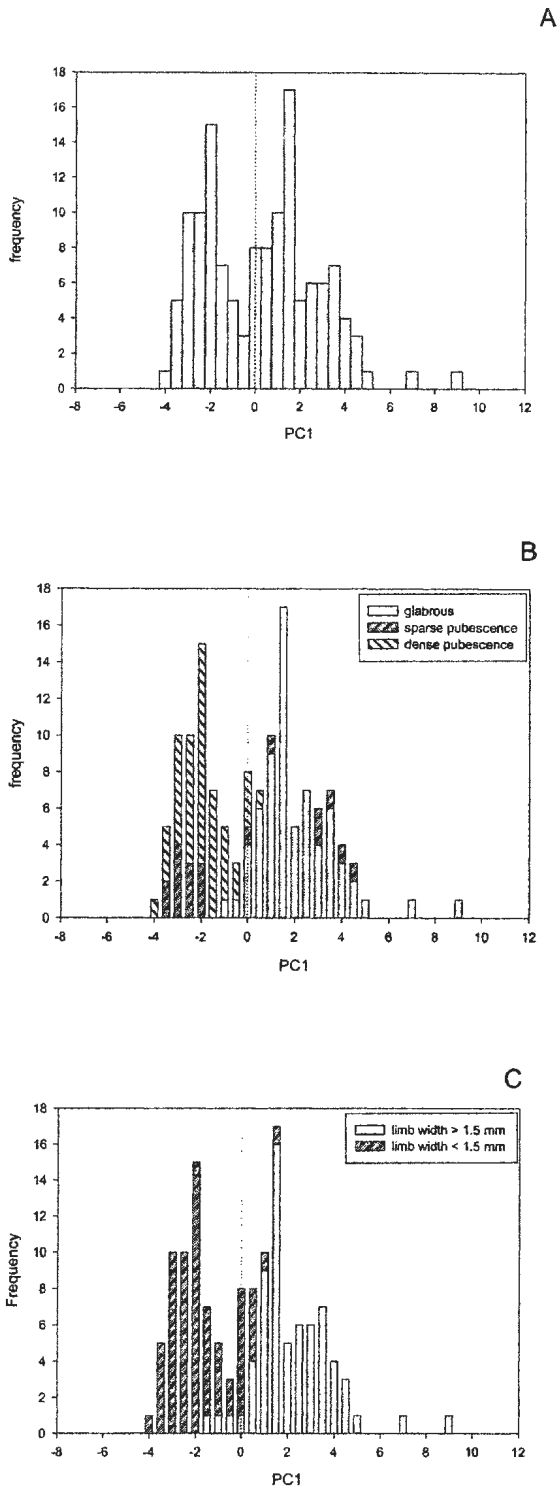

Fig. 1.6. Frequency histograms of the first principal component. (a) Species not coded. (b) coded for degree of pubescence. (c) coded for limb width.

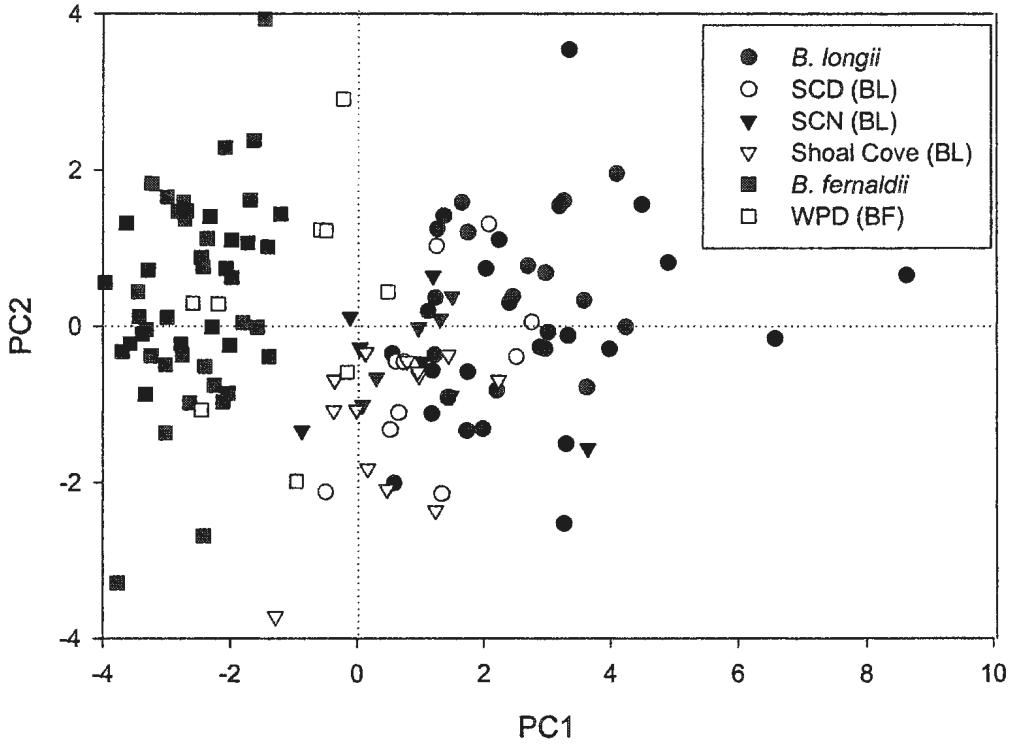

Fig. 1.7. Scatterplot of the first two Principal Components. Each point represents an individual. Coded for 4 sites (Sandy Cove (SCN, SCD), Shoal Cove, and Watt's Point). BL = *B. longii*, BF = *B. fernaldii*. Only populations containing intermediates are coded.

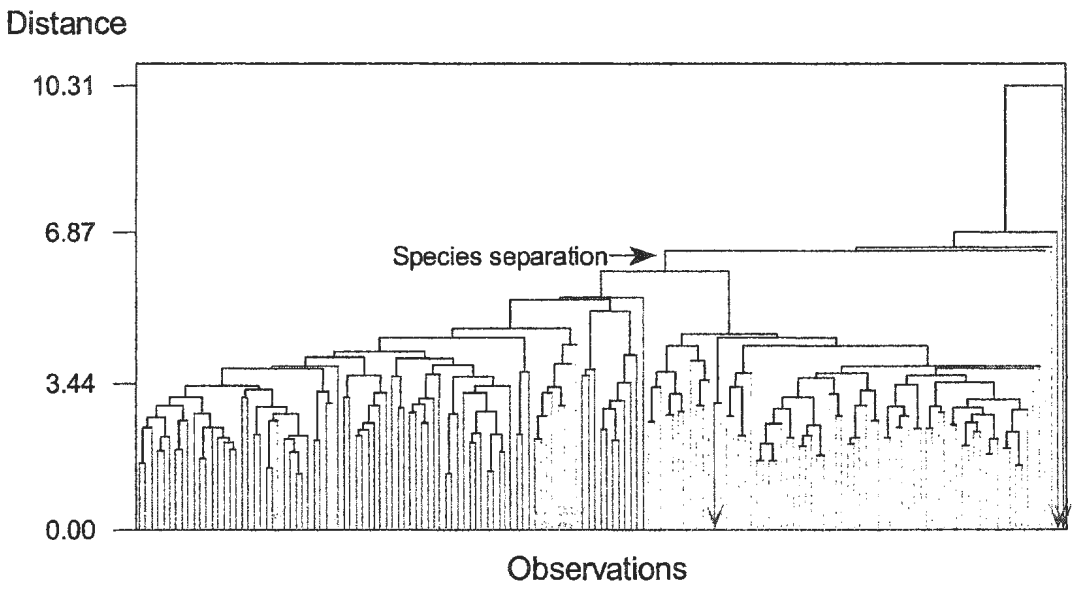

Fig. 1.8. Cluster analysis of floral characters for individuals of *B. longii* (solid lines) and *B. fernaldii* (dotted lines). Separation of species determined by presence of 2 distinct clusters branching at point indicated (by arrow) on graph.

Chapter 2. Reproductive biology of two closely related rare plant species (*Braya longii* and *B. fernaldii*): Implications for conservation management

2.1 Introduction

Understanding the reproductive biology of rare plant species is essential to their management and long-term preservation. There are usually fewer populations among rare species to supply genetic information for future generations (Holsinger, 1991). The reproductive strategy of a species affects its effective population size (N_e), the distribution of genetic variation and hence the design of sampling and management strategies for conservation (Menges, 1991; Given, 1994), such as *ex situ* conservation and reintroduction (Ritland and El-Kassaby, 1985). Breeding systems structure population genetic diversity, transmit diversity across generations and determine the rates of loss of that diversity (Ritland, 1989). Therefore, determining the breeding system of a rare species of plant should be a priority for conservation biologists. The breeding biology of a species also significantly influences the pattern of genetic diversity within and among populations (Loveless and Hamrick, 1984). For example, predominantly selfing species have lower genetic diversity and higher amounts of population differentiation (Barrett and Husband, 1990), whereas outcrossing plant species generally show higher levels of genetic diversity and less differentiation among populations (Van Dijk *et al.*, 1988). Highly autogamous species require the protection of larger numbers of populations in order to capture the greatest range of genetic diversity (Neel *et al.*, 2001). This information has bearing on conservation priorities for *in situ* conservation and on sampling design (Brown and Briggs, 1991).

The breeding systems of *B. longii* and *B. fernaldii* are thought to be primarily autogamous (Harris, 1985). Floral features such as their uniform white flower color and small flower size suggest a selfing breeding system in both species. However, detailed studies of morphological variation between the species (Chapter 1) indicate that *Braya longii* has a greater number of pollen grains per anther, a greater pollen: ovule ratio and a greater degree of herkogamy than *B. fernaldii,* suggesting a greater outcrossing potential. Harris (1985) tested only a single population (exact location unknown) of each species and determined from a greenhouse study that they were self-fertilizing. The nearly 100% fruit set and seed set found in the study also supports an autogamous breeding system. However, the amount of variation in breeding systems within and among populations is unknown.

The consequences of an autogamous breeding system can diminish genetic diversity because selfing may restrict heterozygosity and gene flow, which may reduce the genetic variation in the species as a whole (Kearns and Inouye, 1993). The ecological and evolutionary consequences of self-fertilization depend strongly on the relative fitness of selfed offspring compared to their outcrossed counterparts (Routley *et al.*, 1999). Autogamy may be advantageous, however, because it allows individuals to produce seed when pollinators are scarce (Lloyd, 1992). This "reproductive assurance" may be an important selective advantage, especially for small, isolated rare plant populations, as seasonal seed production is often limited by pollen availability (Burd, 1994). Plants may respond to varying environmental factors via mechanisms that optimize reproductive output (Culley, 2002). For example, the production of less costly cleistogamous flowers

can appear when pollinators are absent or under limiting resource conditions (Culley, 2002).

In morphological terms, cleistogamy is the most extreme form of autogamy (Porras and Munoz, 2000). Since cleistogamous flowers never open and self-pollination occurs in a bud-like stage that is much reduced compared to a 'normal' flower (Berg and Redbo-Torstensson, 1998), there is no potential for outcrossing. Petals are often rudimentary or completely missing, stamens are reduced in size with few pollen grains (Ruiz de Clavijo and Jimenez, 1993). Cleistogamous flowers are considered to have evolved from chasmogamous flowers (Lord, 1981). Cleistogamy is expected to have significant effects on the genetic structure and conservation management of a rare species.

The major negative effect of self-fertilization on plant fitness is inbreeding depression, defined as the relative reduction in fitness of selfed offspring compared with outcrossed offspring (Barrett and Kohn, 1991). However, the effect of inbreeding depression on small plant populations is controversial. If populations have a long history of inbreeding or when selfing is induced by small population size, little inbreeding depression is expected (Barrett and Kohn, 1991) because there is strong selection against the deleterious genes expressed as homozygotes (Silvertown and Lovett-Doust, 1993). In other words, inbreeding for several generations in populations may actually purge the genetic load and the differences between selfed and outcrossed progenies might be less in smaller populations (Briggs and Walters, 1997). This has been refuted, however, in recent studies that show unexpected high inbreeding depression in several selfing species

(Eckert and Barrett, 1994; Husband and Schemske, 1996).

Although inbreeding may cause concern for small plant populations, outcrossing does not always increase fitness. Outbreeding depression, defined as fitness decline upon outbreeding, may also be a concern for rare plants (Fischer and Matthies, 1997). Outbreeding depression commonly occurs in crosses between closely related species or geographically separated populations of a single species (Sobrevil, 1988). Outbreeding depression may also occur in crosses within single populations (Waser and Price, 1983) where plants in one area become locally adapted and suffer lower fitness upon crossing with plants from other areas (Waser and Price, 1989).

Hybridization with closely related species can have the same effects as outbreeding depression, and therefore may threaten the integrity of rare species. Interspecific hybridization occurs commonly in many groups of plants (Soltis and Gitzendanner, 1999) but may have substantial negative impact on rare plant species (Levin *et al.*, 1996). Hybridization has contributed to the extinction of many species (Rhymer and Simberloff, 1996). Hybrid plants may compete with parental species for resources or habitat, and/or may be more susceptible to pests and therefore increase attack on the parent plants. When hybridization occurs, hybrid seeds are produced at the expense of selfed seeds which can have an impact on reproductive output (Soltis and Gitzendanner, 1999).

Braya longii and *B. fernaldii* have recently been found growing in close proximity to each other (pers. obser., Hermanutz *et al.*, 2002), which has the potential to result in hybridization. The close proximity of the two species is mainly due to anthropogenic

disturbances such as the removal and deposition of gravel from one location to another (pers. obser.). Historically, the two species did not grow at the same locations (Fernald, 1926). Contact between species is more likely to occur in anthropogenically disturbed habitats and this may alter competitive relationships, and increase the overall likelihood of hybridization (Anderson, 1948). Therefore potential for hybridization must be assessed for *B. longii* and *B. fernaldii* to prevent potential loss of either parental species.

This study compares the reproductive biology of *B. longii* and *B. fernaldii,* and its implications for conservation management. The objectives of this study were (1) to determine the potential breeding systems of *B. longii* and *B. fernaldii*; (2) to examine inter- and intra-specific differences of both species in breeding systems; (3) to determine levels of inbreeding and outbreeding depression and their effects on the long-term viability of these species; (4) to investigate potential cleistogamy in both species; (5) to determine hybridization potential and its effect on the conservation management of both species.

2.2 Methods

Study Populations

Fieldwork was conducted during July - August of 2000 in 7 braya (3 *B. fernaldii* and 4*B. longii*) sites, and in 5 *B. longii* sites during July – August 2001 on the Great Northern Peninsula of Newfoundland. Data for the cleistogamy study were collected from 10 braya sites (4 *B. fernaldii* and 6 *B. longii*) in 2001 (Tables 2.1 and 2.2).

Study Populations 2000: To determine the level of differentiation among populations, study sites were chosen to encompass the full geographic range of each

species. Originally the most southerly population of *B. fernaldii* (Port au Choix) was to be included in breeding system studies, but due to the unusually warm spring, the plants were past the flowering stage by the initiation of the study. Therefore, Anchor Point was chosen to represent the most southerly population, Burnt Cape was chosen as the most northerly population and Watt's Point was chosen as the central population (Table 2.1; Fig. 2.1). These sites occur over an area of about 100km.

Only three sites were chosen for *Braya longii* because at the time of the experiments (July, 2000), only three populations were known to exist (Fig. 2.2) (Hermanutz, 1998). Sites for *B. longii* included Sandy Cove (gravel crusher and airstrip) and Yankee Point (Table 2.2; Fig. 2.2). These sites are located within a 3 km range.

Study Populations 2001: Only *B. longii* was studied in 2001. Four populations of *B. longii* were examined (Table 2.2; Fig. 2.2), all populations known at the time were included in the study with the exception of Anchor Point where plants were too few in number and too small to use in the experiment. Buds are smaller in *B. fernaldii* than *B. longii*, which makes hand pollinations more difficult. Also, anthers dehisced earlier in *B. fernaldii* with some potential bud-pollination occurring (pers. obser.), therefore *B. fernaldii* was excluded in the 2001 field season.

Timing of Anthesis and Stigma Receptivity

Preliminary observations on *ex situ* plants growing under common garden conditions at Memorial University of Newfoundland Botanical Garden in St. John's, Newfoundland indicated there were species-specific differences in timing of anthesis and stigma receptivity. Therefore to determine optimal bud size and timing for hand-pollinations,

timing of anthesis and stigma receptivity were tested at three bud sizes (upper limits given) for each species: "small" (BL: 1.5 mm, BF: 1.3 mm), "medium" (BL: 1.8 mm, BF 1.6 mm), and "large" (BL: 2.1 mm, BF: 1.8mm). Several populations of each species were tested for timing of anthesis (Tables 2.1 and 2.2). Ten or more buds from each size class within each population were chosen at random and removed and examined under a stereoscope at 40 X magnification. Positive results for anthesis were given if any of the six anthers had dehisced.

Stigma receptivity was determined visually, using a stereoscope at 40X magnification. Presence of elongated papillae on the stigma surface was interpreted as a receptive stigma. This was verified using the benzidine enzymatic reaction (using a freshly made benzidine solution (1% benzidine in 60% ethanol: hydrogen peroxide: water, 4:11:22v/v/v)) (Kearns and Inouye, 1993). Ten or more buds from each size class within each population were tested for stigma receptivity. The same populations (and buds) were tested for stigma receptivity as was done in the tests for timing of anthesis (Table 2.1 and Table 2.2).

Pollen Viability

In order to perform hand-pollinations, the pollen used from other plant populations must be viable. Braya populations on the Great Northern Peninsula are geographically isolated; therefore transport of pollen from one site to another was necessary and sometimes required up to one day of storage. Therefore to determine how long pollen grains remained viable, pollen viability tests were performed during the summer of 2000 using Alexander's stain (Kearns and Inouye, 1993). Five plants from each population used in

the pollination experiments (Tables 2.1 and 2.2) were tested for pollen viability. Several anthers were picked per plant, during daylight hours and left at room temperature in dark storage for periods from one day to one week. Anthers were crushed and pollen was counted at 100 X magnification, using a compound microscope. The percent viable pollen was scored for up to 200 pollen grains for each anther tested.

Hand Pollinations

To determine the variation in breeding systems between species and among populations of each species, experimental hand pollinations were performed following the protocols of Kearns and Inouye (1993). Field pollination experiments were performed *in situ* (Tables 2.1 and 2.2). Ten plants from each population were tagged based on size (>8 scapes) and number of older buds present on the day of pollination (>2 per scape). Each of the eight pollination treatments were performed on each plant, therefore eight scapes were needed. The buds were gently opened with forceps, anthers were removed for those treatments requiring emasculation (Table 2.3), and placed in a labeled vial along with extra anthers harvested from other flowers on the same plant for pollination experiments. Buds were randomly allocated to one of eight treatments (Table 2.3). Each treatment was replicated twice, on different scapes, on each plant. Apomixis tests were not performed on field populations, as greenhouse tests indicated that apomixis does not occur (0 of 40 treated buds produced fruit).

Fewer treatments were performed in 2001 (Table 2.3) but the methods for pollination were the same. To prevent loss of fruits to insect herbivores in 2001, plants

were treated with an insecticide (Cygon® 2E) three days prior to hand-pollinations and once per week for three weeks after pollinations.

The donor pollen was transferred to the stigma with toothpicks, which were discarded after each use. A toothpick was placed into a vial containing anthers, the anthers were gently crushed and the pollen remained attached to the toothpick. Pollen was added only once as greenhouse tests indicated sufficient pollen (> 25 grains: 10-16 ovules) adhered at time of deposition. Pollination treatments were differentiated using different coloured thread tied to individual flower buds. The entire treated scape, with the exception of the open-pollinated control, was then bagged using fine mesh bridal veil and secured at the bottom with a string. In some cases, thin bamboo skewers were used to support the scape due to the extreme windy conditions characteristic of the Northern Peninsula. Pollen used for the crosses was kept in the dark at room temperature for up to three days, during which it remained viable (see pollen tests). The treated scapes remained bagged until fruits were mature. At maturity, treated siliques were harvested, and fruit set (number of treated buds that produced fruit/ number of buds treated) and seed-set (number of mature seeds/total number of ovules) as well as seed weight (up to 5 sets of 5 seeds weighed) was determined for each silique within each treatment.

At harvest, the amount of insect damage by the diamondback moth (*Pleutella xylostella*) was determined by the presence or absence of insect holes in the siliques (Hermanutz and Parsons, 2002). There were diamondback moth larvae and adults observed in all populations tested. In some cases the larvae had pupated within the bridal veil bag, where the adults were then trapped.

The diamondback moth is a small, brown, introduced moth that is native to the southern United States. It is a widely distributed pest of cruciferous crops. The moths are not known to over winter in Newfoundland, and are thought to recolonize each spring via wind currents from the United States (P. Dixon, pers. comm.).

Inbreeding and outbreeding depression

To assess inbreeding and outbreeding depression, fitness components (seed set and weight) were analyzed. Inbreeding depression was evaluated by determining if seed set and seed weight was significantly lower for the selfing treatment (geitonogamy) than for the within population outcross.

Outbreeding depression was evaluated by determining if seed set and/or seed weight was significantly lower for outcrossed treatments (< 10m, > 10m and interpopulation outcross) than for the selfed treatment (geitonogamy).

Cleistogamy

Five to ten buds (of various sizes) were haphazardly removed from 10 – 15 plants in each field study population, stored in FAA (formalin: acetic acid: 70% ethanol 5:5:90 v/v/v) for 48 hours, and subsequently transferred to 70% ethanol until measurements were done (Kearns and Inouye, 1993). Buds were dissected under a stereoscope using forceps. The image was captured by a video camera mounted on the stereoscope using the Snappy Video Snapshot (Version 3.0) program. The UTHSCSA Image Tool (Version 2.0 for Windows) was used to take measurements of all the buds. A total of 13 characters were measured (Table 2.4).

Based on organ development inside the bud, the buds were differentiated into four functional stages (Table 2.5). Five to ten carpels were removed from buds allocated to each stage and stored in ethanol until pollen tube analysis was done. Tissues were softened and cleared for two hours using 1N NaOH. After rinsing with distilled water, carpels were stained using decolourized aniline blue (aniline blue dye in K_2HPO_4) for 24 – 48 hours. The carpels were then sliced in half using a scalpel and squashed in decolorized aniline blue on a slide. Slides were viewed at 100 X – 200 X magnification under an epifluorescence microscope (Kearns and Inouye, 1993). Fluorescence microscopy illuminates callose plugs that are deposited by pollen tubes, allowing the observer to follow tubes from the stigma into the ovary (Kearns and Inouye, 1993). The number of pollen grains on the stigma surface, the number of germinated grains, the number of pollen tubes present in the style and the number of pollen tubes present in the ovary were counted (Table 2.4).

Statistical Analysis

Data from the hand pollination experiment were analyzed in several ways. As fruit set was based on the presence or absence of fruit, Binary Logistic Regression was used (Kent and Coker, 1992). Binary Logistic Regression allowed for the comparison of fruit set between species and among populations within species (G-statistic), and also for comparison of all experimental hand-pollination treatment results to the fruit set results of the open-pollination control (z-value). The G-statistic tests the null hypothesis that all of the coefficients associated with the predictors equal zero (i.e. tests that all slopes are zero) (Minitab, 2000). The z-value tests if the parameters are zero (i.e. it is used to test the

hypothesis of a difference between two means) (Minitab, 2000). One Way ANOVAs were used to determine if there were significant differences in pollen viability, stigma receptivity, timing of anthesis and among population variation in cleistogamy, as well as seed set and seed weight for hand-pollination treatments between species and among populations. The Bonferroni correction method for multiple comparisons was used to compare treatments between species and among populations where p-values less than 0.006 are significant for the year 2000 and p-values less than 0.008 are significant for the year 2001 treatments. When comparing manipulated and non-manipulated buds in 2000 and 2001, all p-values less than 0.05 are significant. Fully Nested ANOVAs were used to identify significant differences in variation among species for the cleistogamy study. Populations were treated as random effects because interest is in the population and not in the individuals. Type III adjusted Sums of Squares were used given that the other terms were already in the model and adjusted sums of squares are the additional sums of squares determined by adding each particular term to the model (Minitab,2000). Minitab (Version 13.31 for Windows) was used to perform all statistical analyses.

2.3 Results

Timing of Anthesis and Stigma Receptivity

In the "small" size class, none of the *B. longii* buds had undergone anthesis (N = 70), which was not significantly different (p = 0.08) from *B. fernaldii*, where anthesis had taken place in 10% of the buds (N = 39) (Fig 2.3 a). In the "medium" size class, 46% of *B. fernaldii* buds (N = 52), but none of the *B. longii* buds (N = 80) had undergone anthesis, resulting in a significant difference ($p < 0.001$). Lastly in the "large" size class,

only 6% of *B. longii* buds (N = 79) had undergone anthesis, which is significantly lower ($p<0.001$) than the 86% found in *B. fernaldii* (N = 50).

The mean percentage of receptive stigmas at each bud size class did not greatly differ between species. In the "small" size class, *B. longii* had 13% of buds with receptive stigmas (N = 69) and *B. fernaldii* 23% (N = 39), which was not significantly different ($p = 0.442$). In the "medium" size class, 60% of *B. longii* buds (N = 80) and 80% of *B. fernaldii* (N = 52) had receptive stigmas, which was significantly different ($p = 0.005$). Finally in the "large" size class there was no significant difference ($p = 0.168$), where 96% of *B. longii* buds (N = 79) and 100% of the *B. fernaldii* buds (N = 50) had receptive stigmas (Fig. 2.3 b).

For *B. longii*, large-sized buds (2.1 mm) were used for hand-pollinations because stigmas are receptive at this size and anthers have not yet dehisced. For *B. fernaldii*, medium-sized buds (1.6 mm) were used, where most stigmas are receptive and most anthers had not yet dehisced. In the field, a magnifying glass was used to help determine if the medium-sized buds met the above criteria. If pollen was visible on the stigma, the bud was not used and a smaller bud (as small as 1.2 mm) was used instead.

Pollen viability. – There was no significant difference (Table 2.6) in pollen viability between species ($p = 0.256$). All viability counts were high (>94%) and proportion of viable pollen ranged from 0.91 – 1.0 for *B. longii* and 0.92 – 1.0 for *B. fernaldii*. Thus, pollen could be allowed to remain at room temperature for up to one week without affecting viability.

Experimental Hand Pollinations

Fruit set. – Many of the experimental crosses were destroyed by the diamondback moth (*Plutella xylostella*). Of the 485 crosses performed on *B. longii* in 2000, 213 (44%) were destroyed by the diamondback moth, while 262 of the 461(57%) crosses performed on *B. fernaldii* were lost. Damage was not as severe in 2001 as an insecticide treatment was used to deter the moths. Of the 565 crosses performed on *B. longii,* 143 (25%) of these were destroyed. Buds that were completely eaten by diamondback moth larvae were removed from the dataset (Table 2.7).

Fruit set was very high in the open pollination controls (Fig. 2.4). The only interspecific difference in fruit set was in the outcrossing control ($p = 0.005$) with *B. longii* setting 64% fruit and *B. fernaldii* setting 18% fruit (Fig. 2.4), indicating that *B. longii* has a higher potential for outcrossing.

For *B. longii* in 2000, all treatments had significantly lower fruit set ($p \leq 0.002$) than the open-pollination control with the exception of the self-pollination control ($p = 0.136$) and the outcrossing control ($p = 0.046$; Table 2.8a). In 2000, *Braya fernaldii* results were similar with all having lower fruit sets ($p \leq 0.006$) when compared to the open pollination control, with the exception of the self-pollination control ($p = 0.065$; Table 2.8b). Both species produced fruit in all treatments in both years (Fig. 2.4 and 2.5). In 2001, *Braya longii* had lower fruit set in all treatments compared to the control ($p< 0.001$), with the exception of the self-pollination control ($p = 0.976$; Table 2.8c).

Population Variability in Breeding System. – Population variation in fruit set among treatments within *B. longii* (Table 2.9) and *B. fernaldii* (Table 2.10) showed contrasting

results. Within *B. longii*, two of the treatments showed differences for fruit set among populations, geitonogamy ($p = 0.001$) and the within population outcross < 10m ($p = 0.003$). The fruit set for the geitonogamous treatment was much lower in the APN (0 ± 0) population, and for the within population outcross <10m (APN: 0 ± 0). This may indicate some among population differences in potential inbreeding depression. For *B. fernaldii*, there was no variation found among populations for any of the treatments, indicating a lower level of population differentiation in potential breeding systems. In 2001, there were significant differences among populations within *B. longii* for two of the treatments; geitonogamy ($p = 0.001$) and the outcrossing control ($p = 0.007$; Table 2.11). SCD fruit set was lowest for the geitonogamy treatment (0.27 ± 0.12), and both APD and Shoal Cove had the lowest fruit set (0 ± 0) for the outcrossing control treatment.

Population differences found for *B. longii* in both years may be due to genetic differences among populations, different levels of inbreeding or outbreeding depression or differences in the amount of insect damage for each population. There were significant differences in the amount of insect damage among *B. longii* populations in 2000 ($p = 0.002$) and 2001 ($p < 0.001$; Fig. 2.6).

Seed set. - In 2000, there were no significant differences between species for any of the hand-pollination treatments (Fig. 2.7 a). However, there were significant differences in seed set for hand-pollination treatments compared to the open pollination control treatment, within both species. The open-pollination control had higher seed set ($p <0.001$) than all other treatments within both species (Fig. 2.7 b, c). As in 2000, within

B. longii there were significant differences in seed set among treatments in 2001 (Fig. 2.8). There was no significant difference in seed set for the outcrossing control ($p = 0.421$), self-pollination control ($p = 0.336$) or the geitonogamy ($p = 0.042$) treatments when compared to the open-pollination control. There was, however, significantly lower seed set for the within population ($p < 0.001$) and interpopulation outcross ($p = 0.001$) and the hybrid pollination ($p = 0.002$) treatments when compared to the open pollination control.

Seed Weight. - In 2000, there were no significant differences in seed weights among treatments found between ($p = 0.448$) or within (*B. longii* $p = 0.540$; *B. fernaldii* $p = 0.784$) species (Fig. 2.9). Similar results were seen in 2001 within *B. longii*, where there were no significant differences in seed weight found among different pollination treatments ($p = 0.218$; Fig. 2.10).

Inbreeding Depression. - For *B. longii* in 2000 there was no significant difference in seed set for the geitonogamy treatment and the outcross < 10m treatment ($p = 0.985$; Fig. 2.7), or for seed weight ($p = 0.841$) (Fig. 2.9). Similar results were seen in 2001, except there was significantly lower seed set for the outcross treatment when compared to the geitonogamy treatment ($p = 0.008$; Fig. 2.8) but no significant difference in seed weight was found ($p = 0.443$; Fig. 2.10) suggesting no evidence for inbreeding depression in *B. longii.*

Within *B. fernaldii* there was no significant difference in seed set ($p = 0.106$) or seed weight ($p = 0.102$) when comparing the geitonogamy and outcross (< 10m)

treatments (Fig. 2.7; Fig. 2.9) also suggesting no evidence for inbreeding depression.

Outbreeding Depression. - For *B. longii* in 2000, the within population outcross treatments (< 10 m and > 10m) and the interpopulation outcross treatment produced some fruits (Fig. 2.4). When seed set was analyzed (Fig. 2.7), there was no significant difference for seed set for the > 10m outcross ($p = 0.107$) or the interpopulation outcross ($p = 0.117$) compared to the geitonogamy treatment. No significant differences in seed weight were found for either treatment ($p = 0.775$ and $p = 0.974$, respectively; Fig. 2.9). There were differences in the 2001 data where the interpopulation treatment produced lower seed set ($p = 0.002$) when compared to the geitonogamy treatment (Fig. 2.8). There was no significant difference in seed weight for the interpopulation treatment when compared to the control ($p = 0.572$; Fig. 2.10). These results suggest some outbreeding depression for *B. longii*. There was lower seed set for the outcross treatments, in 2001, for both the within population and interpopulation treatments when compared to the geitonogamy treatment.

Braya fernaldii produced fruits in all treatments (Fig. 2.4). There was no significant difference in seed set or seed weight in the > 10m within population outcross treatment ($p = 0.144$; Fig. 2.7 and Fig. 2.9). However, there was lower seed set for the interpopulation outcross when compared to the geitonogamy treatment ($p = 0.026$), but no significant difference in seed weight ($p = 0.092$). This may be evidence for some outbreeding depression in *B. fernaldii.*

Insect Damage by Diamondback Moth. - Agrawal et al. (1999) found that damage to the

foliage of wild radish resulted in increased release of indole glucosinolates. The diamondback moth is stimulated to feed by many glucosinolates (Talekar and Shelton, 1993). There was concern that the crossing interventions (those treatments involving emasculation (Table 2.3)) "attracted" insects, and therefore those treatments sustained more damage. When those treatments were compared, within both species, there was a significantly greater (*B. longii* $p = 0.048$; *B. fernaldii* $p < 0.001$) amount of insect damage to buds that were opened and emasculated compared to those that were (Table 2.12). There was a much greater difference in the *B. fernaldii* treatments. The smaller bud sizes used in the hand pollination experiments for *B. fernaldii* could have been a contributing factor to these differences.

The same analysis was done for 2001 data but there was no significant difference in the amount of insect damage for buds receiving different hand-pollination treatments ($p = 0.679$). The observed differences in insect damage between years is likely a result of the application of insecticide, which took place only in 2001.

Cleistogamy

Observations in the field during the summers of 2000 and 2001 suggested that bud-pollination occurred in at least one population of *B. fernaldii* - Burnt Cape (pers. obser.). At each functional bud (closed) stage, *B. longii* buds were significantly larger ($p \leq 0.002$) than those of *B. fernaldii* as were the lengths of the anthers ($p \leq 0.003$; Table 2.13). At all functional bud stages, *B. fernaldii* had greater numbers of pollen grains present on the stigma and germinated on the stigma, as well as greater numbers of pollen tubes in the style and ovary than *B. longii* (Fig. 2.11). None of these were statistically significant

except at bud stage 4 where there were significantly greater numbers of pollen grains present on the stigmas in *B. fernaldii* than on *B. longii* ($p = 0.002$). Although the results are not statistically significant at the earlier functional bud stages, the fact that some individuals of *B. fernaldii* have pollen present on stigmas within closed buds suggests that bud-pollination was occurring.

The numbers of individuals displaying cleistogamy is very variable within each species. Since only one population of *B. fernaldii* was suspected of exhibiting cleistogamy, populations within each species were analyzed (see Appendix 2). Burnt Cape was the only population that consistently showed evidence of bud-pollination at all functional bud stages. At functional stage 1, only one individual from one population (Burnt Cape) was bud pollinating, therefore there was no significant among population difference found ($p = 0.227$). The same was true for functional stage 2 with two individuals from Burnt Cape were bud-pollinating. At functional bud stage 3, while no populations of *B. longii* had pollen present on stigmas, three of the four *B. fernaldii* populations did. Burnt Cape had the greatest average number of pollen grains present on the stigma although this difference was not found to be significant ($p = 0.661$). The same trend was found for functional bud stage 4.

The average number of pollen grains per stigma was low in all cases because within population variation was high. Pollen grain numbers ranged from zero to 52 and most individuals in all of the populations examined had no pollen grains on their stigmas. Therefore, although cleistogamy occurs only in *B. fernaldii*, and levels among populations vary, the amount of temporal variation is unknown, as are the causes of this

variation.

2.4 Discussion

Braya longii was found to be primarily autogamous with potential for outcrossing. On the otherhand, *B. fernaldii* was found to be completely autogamous with little or no potential for outcrossing. Cleistogamy was limited to a single population of *B. fernaldii.*

This breeding biology study was negatively affected by the amount of diamondback moth (*Plutella xylostella*) damage the plants received in both field seasons (2000 and 2001). Despite this insect damage, plants in all treatments produced some fruit and seeds although both fruit set and seed set were low compared to the open-pollination control. Tests showed that pollen was viable and stigmas were receptive for all bud sizes used in the hand pollinations, therefore they were not limiting fruit or seed production. If there was damage caused by the treatment of buds (emasculation, or opening buds with forceps) it might have affected the formation of fruit resulting in lower fruit set and seed set. Mussury and Fernandes (2000) found in outcrossed pollination tests for canola (*Brassica napus*) that the size and number of seeds were inferior in relation to the autogamy test and natural pollination tests. They concluded that this was due to the manipulation of the bud during emasculation and pollen deposition (Mussury and Fernandes , 2000). A similar problem may explain the results of the present breeding system study of *B. longii* and *B. fernaldii.*

Although Harris (1985) found *B. longii* to be completely autogamous, the present experiments provide evidence that there is potential for outcrossing. The potential for outcrossing was determined for all populations of *B. longii* with the exception of Shoal

Cove, however the realized level of outcrossing among populations is unknown. Breeding system experiments did confirm Harris' conclusions for *B. fernaldii* which appears to be totally autogamous with the exception of Watt's Point population, which had a high potential for outcrossing, similar to the experimental results found for *B. longii*.

This breeding system study supports the results found in the floral morphology study (Chapter 1). *Braya longii* plants have larger flowers as measured by limb width (1.75 mm ± 0.03), a greater degree of herkogamy (0.57 mm ± 0.02), a greater number of pollen grains per anther (499 ± 18.4) and a greater pollen:ovule ratio (37.95 ± 18.4) than *B. fernaldii* (1.12mm ± 0.02; 0.21 mm ± 0.02; 269.2 ± 11.7; and 19.91 ± 1.25, respectively) supporting the finding of greater potential for outcrossing.

Although the potential for outcrossing exists within *B. longii*, it is unknown if outcrossed pollen successfully competes with geitonogamous pollen on stigmas under normal field conditions (in the outcrossing control, anthers were removed, therefore there was no geitonogamous pollen on the stigmas). What proportion of the open pollinated individuals are actually outcrossed is unknown. Therefore, outcrossing rate in *B. longii*, cannot presently be estimated. Appropriate molecular markers should be used to estimate outcrossing rate under field conditions. No pollinators were observed during daylight hours (8 am to 9 pm) in the field in 2000 or 2001. If outcrossing does occur naturally, it is most likely via small insects such as mosquitoes, or the wind. Williams (1985) observed in a canola (*Brassica napus*) plantation that wind and bees were major pollen vectors, while Williams (1978) found wind also promoted self-pollination in the field. Given the

windy conditions typical of the Northern Peninsula, wind is most likely aiding in the dispersal of pollen in braya. The wind "shakes" the scapes, and at the same time, may shake pollen within flowers or among flowers of an inflorescence (pers. obser.).

Population Differentiation in Breeding Systems

Breeding systems (outcrossing and selfing rates) can differ among populations within a species (Kearns and Inouye, 1993). Differences in breeding systems or even potential breeding systems may affect a species genetic structure. There were no differences in fruit set among populations of *B. fernaldii* for any of the treatments, suggesting that there is limited genetic variation in breeding system across its range. However, there were fruit set differences among populations of *B. longii* in both 2000 and 2001. The population differences found within *B. longii* may reflect potential differences in outbreeding depression, outcrossing potential or differences in the amount of insect damage each population received.

The differences found within *B. longii* could therefore be important when implementing management strategies for this species. Population differentiation can also have impacts on *in situ* conservation and collection (Brown and Biggs, 1991). When there are high levels of population differentiation, increasing the size of *in situ* populations is not as important as conserving the genetic variation among populations. Therefore all populations must be conserved to maintain existing levels of genetic variation. Seed collection for *ex situ* seed storage will have to be sampled from all populations in order to preserve the entire range genetic variation contained within the species.

Evidence of inbreeding and outbreeding depression

There is no evidence to support inbreeding depression in *B. longii* or *B. fernaldii*. These results support the theory that when a species has a long history of inbreeding, there may be strong selection against deleterious genes expressed by homozygotes, resulting in little inbreeding depression (Silvertown and Lovett-Doust, 1993). Studies by Holtsford (1989) on inbreeding depression in three populations of *Clarkia tembloriensis* with different selfing rates also support this theory and showed that the most selfing population displayed the least amount of inbreeding depression.

There was evidence for outbreeding depression in both species. This was predicted because the species are autogamous and there is likely to be limited gene flow between isolated populations. Reduced fitness upon outbreeding commonly occurs in crosses between isolated populations, and even crosses within a population (Waser and Price, 1989). The risks of outbreeding depression must be taken into account when attempting re-introductions or translocations.

Cleistogamy

The present study supports field observations that bud pollination or pseudo-bud pollination (having highly reduced flowers that may not open and generally self-pollinate in the bud (Fishman and Wyatt, 1999)) is also occurring in at least one population of *B. fernaldii* (Burnt Cape). This is similar to what was found in the two races (one virtually cleistogamous and one outcrossing) of *Arenaria uniflora* (Fishman and Wyatt, 1999). The self pollinating populations were not herkogamous, flowers were much smaller (< 4

mm vs. 1 cm), and the numbers and display time (flowers open 1 day vs. 2 weeks) of floral organs were reduced compared to the outcrossing populations (Fishman and Wyatt, 1999).

The true degree of cleistogamy may have been more obvious if buds were collected earlier in the season. Observations in the field suggest that bud pollination is occurring, at least at Burnt Cape, in the buds that open first, lower on the flowering scape. Unfortunately, the buds in this study were sampled using only buds near the top of the flowering scape (buds that had developed later in the season). Future studies should sample from all regions on the flowering scapes to determine if there is temporal variation in expression of cleistogamy.

Plants respond to variation in the surrounding environment and these responses often involve mechanisms that promote reproductive assurance (Culley, 2002). Reproduction can be affected by differences in pollinator availability or seed predation (Schoen and Lloyd, 1984) as well as changes in light, moisture, temperature or nutrient availability (Upof, 1938). A temporal switch from chasmogamous to cleistogamous flowers may correspond with these changes (Culley, 2002). The cleistogamous (or pseudo-cleistogamous) flowers found within *B. fernaldii* may be a result of responses to local environment. The single population (Burnt Cape) that consistently displays cleistogamy at all bud stages is isolated from all other *B. fernaldii* populations, suggesting that local environmental conditions could contribute to expression of this trait. The cleistogamy at Burnt Cape may be induced by the colder environmental conditions; this would have to be tested experimentally. However, the presence of cleistogamy at bud

stage 3 in three of four *B. fernaldii* populations sampled suggests that the switch from chasmogamy to cleistogamy may not be environmental.

Hybridization Potential

Braya longii and *B. fernaldii* are very closely related and in a few areas, grow in close proximity to one another. There have also been concerns for potential hybrids because of intermediate phenotypes found in the field (Chapter 1). If there are hybrids present in populations, they could potentially persist because of self-pollination. Hybridization is deleterious because of loss of biodiversity and could lead to the replacement of parental form by hybrid individuals (Ellstrand and Elam, 1993). If pollination can occur via wind or insects in natural populations, as it did in the outcrossing control for *B. longii,* the potential for hybridization must be assessed. In 2000 and 2001, the hybridization treatment for *B. longii* did result in some fruit being set (30% and 13% fruit set respectively). Although in both years, seed set was significantly lower in the hybridization treatment when compared to the open-pollination control, there were mature seeds produced. Although *B. fernaldii* plants produced some fruit (25% fruit set), there were no seeds produced in the hybrid pollination treatment. Therefore there is potential for hybridization with *B. longii* as the maternal parent and *B. fernaldii* as the pollen parent.

Implications for Conservation Management

Knowledge of breeding systems has potential application in the management of rare and endangered species to conserve their genetic variability (Kearns and Inouye, 1993) and is essential to the long-term recovery and conservation strategy of rare plants.

The breeding system of *B. longii* is primarily autogamous with the potential for outcrossing while the breeding system of *B. fernaldii* is completely autogamous. There are few populations of these rare *Braya* species and it is likely that they have had a long history of inbreeding. These species probably benefited from the reproductive assurance of autogamy given their small distributions and lack of available pollinators on the Great Northern Peninsula. Therefore, it was predicted that there would be little inbreeding depression, as was found in this study, because inbreeding for several generations can diminish differences between selfed and outcrossed progenies (Briggs and Walters, 1997).

However, outbreeding depression is evident in both *B. longii* and *B. fernaldii*. Consequences of outbreeding depression include isolation of populations and population differentiation (Charlesworth and Charlesworth, 2000). This may be the case for *B. longii* and *B. fernaldii* as there was some evidence of population differentiation. Therefore conservation efforts for these rare *Braya* species may need to focus on conserving the genetic variation found among populations. Protection should include examples of all populations because conservation of a single population would not encompass the genetic diversity contained within the species.

A potential management tool to increase genetic variation within selfing populations would be to perform outcrossing hand-pollinations or stimulation of natural outbreeding between populations (Barrett and Kohn, 1991). However, before these strategies can be considered safe, pollen must be chosen carefully because of the potential for outbreeding depression. Pollen should be used from plants at less than 10 m distance

to avoid decreased fitness of offspring caused by outbreeding depression. Also, populations should not be mixed to avoid outbreeding depression caused by interpopulation outcrosses.

The evidence presented, suggesting the potential for hybridization between the two species, will also affect management strategies. Hybridization may lead to the extinction of rare species, by inhibiting the ability of plants to reproduce, and to resist damage by pests and pathogens (Soltis and Gitzendanner, 1999). When implementing conservation efforts such as re-introduction, recovery teams must be aware of hybridization. Thus, reintroduction should be done in areas where other species (with the ability to form hybrids) do not occur. Conservation efforts should be directed to prevent hybridization and further loss of both parental species.

In conclusion, future conservation management strategies should consider the results of this breeding system study. Preservation of genetic variation both within and among populations is key to ensuring long-term preservation of both species. The potential differences in outcrossing rates are important, as these differences may be the key for restoring diversity within populations. Therefore conservation efforts must focus on conserving sufficient variation within all populations. The potential for hybridization may also have considerable impact on restoration and reintroduction strategies and should be considered in future management strategies.

2.5 References

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Table 2.1. Disturbance regimes, size, and indication of study for populations of *Braya fernaldii*. Population size estimates from Hermanutz *et al.* 2002.

				STUDIES BY POPULATION				
Population	**Symbol**	**Disturbance Regime**	**Pop. Size**	**Pollination Field 2000**	**Anthesis**	**Stigma Receptivity**	**Pollen viability**	**Cleistogamy**
Anchor Point (West of hwy)	Anc.Pt.	Natural	650	✓	✓	✓	✓	✓
Anchor Point (East of hwy)	St.Bb	Natural	250					✓
Watt's Point	WPD	Anthropogenic	1050	✓	✓	✓	✓	
Cape Norman	CNN	Naturall	150		✓	✓		✓
Burnt Cape(South)	BCD	Anthropogenic	950	✓	✓	✓	✓	✓

Table 2.2. Disturbance regimes, size, and indication of study for populations of *Braya longii*. Population size estimates from Hermanutz *et al.* 2002.

				STUDIES BY POPULATION					
Population	**Symbol**	**Disturbance Regime**	**Pop. Size**	**Pollination Field 2000**	**Pollination Field 2001**	**Anthesis**	**Stigma Receptivity**	**Pollen viability**	**Cleistogamy**
Anchor Point	Anc.Pt.	Natural	50					✓	
Sandy Cove (crusher)	SCD	Anthropogenic	500	✓	✓	✓	✓	✓	✓
Sandy Cove (crusher)	SCN	Naturally	600		✓	✓	✓	✓	✓
Sandy Cove (airstrip)	APD	Anthropogenic	2400	✓	✓	✓	✓	✓	✓
Sandy Cove (airstrip)	APN	Natural	900	✓	✓	✓	✓	✓	✓
Shoal Cove	Sho.Co.D	Anthropogenic	70		✓	✓	✓		✓
Yankee point	YPD	Anthropogenic	1600	✓	✓	✓	✓	✓	✓

Table 2.3. Description of experimental crosses in field (2000 and 2001) studies for *B. longii* and *B. fernaldii*.

Cross	Description	Emasculated	Bagged	Field 2000	Field 2001
Open Pollination	control			✓	✓
Outcrossing (by pollinators)	control	✓		✓	✓
Self-pollination	control		✓	✓	✓
Geitonogamy	pollen from flowers of same plant	✓	✓	✓	✓
Close Outcrossing	pollen from plant at 1 m distance (within population)	✓	✓	✓	
Far Outcrossing	pollen from plant at >10 m distance (within population)	✓	✓	✓	
Outcrossing	pollen from plant within population	✓	✓		✓
Interpopulation Outcrossing	pollen from plant of different population	✓	✓	✓	✓
Hybrid pollination	pollen from plant of different species	✓	✓	✓	✓

Table 2.4. Description of bud characters measured. All measurements were taken to the nearest 0.01 mm unless otherwise noted.

Character	Description
1 Bud Length	From base of bud to tip of bud
2 BudWidth	Width of bud at widest part
3 Petal Length	From base of claw to tip of limb (one petal chosen haphazardly from the 4)
4 Stamen Length	Measured from base of filament to tip of anther one stamen chosen haphazardly from the 4 tall anthers
5 Anther Length	Using same stamen, measured from base of anther to tip of anther
6 Carpel Length	Measured from base of ovary to tip of stigma
7 Style Length	Measured from tip of ovary to base of stigma
8 Herkogamy	Tip of stigma – tip of anther L (absolute value)
9 Stigma Receptivity	Presence or absence of papilli on stigma surface
10 # of pollen grains on stigma surface	Number of pollen grains on stigma surface counted (using epiflourescence microscope)
11 # of germinating pollen grains on stigma surface	Numbers of germinating pollen grains on stigma surface counted using epifluorescence microscope and decolorized aniline blue
12 # pollen tubes in style	Numbers of pollen tubes in style counted using epifluorescence microscope and decolorized aniline blue
13 # pollen tubes in ovary	Numbers of pollen tubes in ovary counted using epifluorescence microscope and decolorized aniline blue

Table 2.5. Descriptions of four functional bud stages used to compare differences in bud development between *Braya longii* and *B. fernaldii*.

Functional bud stage	Description
Stage 1	Gynoecium is less than 50% of its final length, stamens are at approx. same height as gynoecium, anthers plump and smooth
Stage 2	Gynoecium is approx. 50 – 75% of its final length, stamens are approx. equal height as gynoecium (or slightly above)
Stage 3	Gynoecium is approx. 100% of its final height, stamens are slightly below height of gynoecium in BL and slightly above in BF. Anthers are 100% of their final size.
Stage 4	bud is just about to open. Stamens are higher than the gynoecium in both BL and BF. Anthers slightly decreased in size.

Table 2.6. Comparison of pollen viability for *B. longii* and *B. fernaldii*. N = number of flowers sampled in population. Population descriptions in Tables 1 and 2.

Braya longii					*Braya fernaldii*				
Pop	**N**	**Mean viability**	**SE**	**Range**	**Pop**	**N**	**Mean viability**	**SE**	**Range**
APD	6	0.94	0.01	0.91-0.97	Anc. Pt	5	0.98	0.01	0.94-1.0
SCD	5	0.97	0.02	0.92-1.0	BCD	6	0.98	0.01	0.96-1.0
SCN	5	0.97	0.02	0.91-1.0	WPD	5	0.97	0.02	0.92-1.0
YPD	6	0.99	0.01	0.95-1.0					

Table 2.7. Numbers of experimental crosses destroyed by the Diamondback moth and numbers remaining that were used for statistical analysis of experimental crosses for *B. longii* and *B. fernaldii* in 2000 and 2001.

	Braya fernaldii			*Braya longii* 2000			*Braya longii* 2001		
Treatment	**# crosses performed**	**# crosses destroyed**	**# crosses remaining**	**# crosses performed**	**# crosses destroyed**	**# crosses remaining**	**# crosses performed**	**# crosses destroyed**	**# crosses remaining**
Open Pollination Control	78	13	65	68	10	58	78	3	75
Geitonogamy	51	27	24	63	26	37	88	23	65
Outcross (< 10 m)	54	37	17	58	21	37	-	-	-
Outcross (>10 m)	44	32	12	57	30	27	-	-	-
Outcross (Within population)	-	-	-	-	-	-	83	25	58
Interpopulation Outcross	105	65	40	82	52	30	88	31	57
Hybrid pollination	55	43	12	55	28	27	80	26	54
Self-pollination (Control)	41	23	18	54	23	31	88	19	69
Outcrossing (Control)	33	22	11	48	23	25	60	16	44
Total :	461	262	199	485	213	272	565	143	422

Table 2.8. Comparison of mean fruit set to the open pollination control for a) *B. longii* in 2000, b) *B. fernaldii* in 2000 and c) *B. longii* in 2001 using Binary Logistic Regression. N = number of buds treated, * = significantly different from open pollination control, p<0.006 following Bonferroni correction method for multiple comparisons.

a)

Treatment	N	Mean Fruit Set	SE	Z	p
Open Pollination Control	58	0.8448	0.048	-	-
Geitonogamy	37	0.5405	0.0831	-3.13	0.002 *
Outcross (< 10 m)	37	0.4054	0.0818	-4.21	<0.001 *
Outcross (>10 m)	27	0.5185	0.0980	-3.06	0.002 *
Interpopulation Outcross	30	0.5	0.0928	-3.29	0.001 *
Hybrid Pollination	27	0.2963	0.0896	-4.60	< 0.001 *
Self-pollination (Control)	31	0.7097	0.0829	-1.49	0.136
Outcrossing (Control)	25	0.64	0.0980	-2.03	0.043

b)

Treatment	N	Mean Fruit Set	SE	Z	p
Open Pollination Control	65	0.8615	0.043	-	-
Geitonogamy	24	0.583	0.103	-2.72	0.006 *
Outcross (< 10 m)	17	0.529	0.125	-2.83	0.005 *
Outcross (>10 m)	12	0.333	0.142	-3.55	< 0.001 *
Interpopulation Outcross	40	0.3250	0.750	-5.19	< 0.001 *
Hybrid Pollination	12	0.250	0.131	-3.87	< 0.001 *
Self-pollination (Control)	18	0.667	0.114	-1.84	0.065
Outcrossing (Control)	11	0.182	0.122	-3.87	< 0.001 *

c)

Treatment	N	Mean Fruit Set	SE	Z	p
Open Pollination Control	75	0.8533	0.0411	-	-
Geitonogamy	65	0.5231	0.0624	-4.07	< 0.001 *
Outcross (within population)	58	0.1897	0.0519	-6.87	< 0.001 *
Interpopulation Outcross	57	0.1930	0.05297	-6.82	< 0.001 *
Hybrid Pollination	54	0.1296	0.0461	-7.05	< 0.001 *
Self-pollination (Control)	69	0.8551	0.0427	0.03	0.976
Outcrossing (Control)	44	0.3182	0.0710	-5.49	< 0.001 *

Table 2.9. A comparison of fruit set (# treated buds/ # treated buds that produced fruits) means among populations of *B. longii* tested in 2000 using Binary Logistic Regression. N = number of buds treated, * = significant p-value (< 0.006) after Bonferroni correction method for multiple comparisons. Population descriptions in Table 2.2.

Treatment	Population	N	Mean Fruit Set	SE	G	DF	p
Geitonogamy	APD	9	0.33	0.17	19.432	3	0.001 *
	APN	7	0	0			
	SCD	13	0.85	0.10			
	YPD	8	0.75	0.16			
Within Population Outcross (< 10 m)	APD	10	0.7	0.15	14.3	3	0.003 *
	APN	10	0	0			
	SCD	10	0.5	0.17			
	YPD	7	0.43	0.20			
Within Population Outcross (> 10 m)	APD	6	0.67	0.21	9.220	3	0.027
	APN	5	0	0			
	SCD	10	0.7	0.15			
	YPD	6	0.5	0.22			
Inter-population Outcross	APD	10	0.6	0.16	11.419	3	0.010
	APN	6	0	0			
	SCD	11	0.73	0.14			
	YPD	3	0.33	0.33			
Hybrid Pollination	APD	8	0.25	0.16	5.456	3	0.141
	APN	5	0	0			
	SCD	10	0.5	0.17			
	YPD	4	0.25	0.25			
Self Pollination Control	APD	12	0.92	0.08	7.008	3	0.072
	APN	8	0.38	0.18			
	SCD	7	0.71	0.18			
	YPD	4	0.75	0.25			
Outcrossing Control	APD	10	0.6	0.16	6.202	2	0.102
	APN	0	-	-			
	SCD	9	0.89	0.11			
	YPD	5	0.4	0.24			
Open Pollination Control	APD	22	0.82	0.08	0.272	3	0.965
	APN	7	0.86	0.14			
	SCD	20	0.85	0.82			
	YPD	9	0.89	0.11			

Table 2.10. A comparison of fruit set (# treated buds/ # treated buds that produced fruits) means among populations of *B. fernaldii* tested in 2000 using Binary Logistic Regression. N = number of buds treated. Population descriptions in Table 2.1. - = no data.

Treatment	Population	N	Mean Fruit Set	SE	G	DF	p
Geitonogamy	Anc Pt	12	0.58	0.15	6.766	2	0.034
	BCD	3	0	0			
	WPD	9	0.78	0.49			
Within Population Outcross (< 10 m)	Anc Pt	9	0.67	0.17	2.490	1	0.288
	BCD	0	-	-			
	WPD	7	0.43	0.20			
Within Population Outcross (> 10 m)	Anc Pt	3	0.33	0.33	0.872	1	0.647
	BCD	0	-	-			
	WPD	8	0.38	0.18			
Inter-population Outcross	Anc Pt	17	0.41	0.12	5.337	2	0.069
	BCD	6	0	0			
	WPD	17	0.35	0.12			
Hybrid Pollination	Anc Pt	5	0.2	0.2	1.764	2	0.414
	BCD	2	0	0			
	WPD	5	0.4	0.25			
Self Pollination Control	Anc Pt	5	0.6	0.25	2.125	2	0.346
	BCD	6	0.5	0.22			
	WPD	7	0.86	0.14			
Outcrossing Control	Anc Pt	3	0	0	6.612	2	0.037
	BCD	5	0	0			
	WPD	3	0.67	0.33			
Open Pollination Control	Anc Pt	24	0.75	0.09	4.366	2	0.113
	BCD	19	0.89	0.07			
	WPD	22	0.95	0.05			

Table 2.11. A comparison of fruit set (# treated buds/ # treated buds that produced fruits) means among populations of *B. longii* tested in 2001 using Binary Logistic Regression. N = number of buds treated, * = significant p-value (< 0.008) after Bonferroni correction method for multiple comparisons. Population descriptions in Table 2.2.

Treatment	Population	N	Mean Fruit Set	SE	G	DF	p
Geitonogamy	APD	15	0.87	0.91	21.655	4	0.001 *
	APN	15	0.54	0.13			
	SCD	15	0.27	0.12			
	Sho.Cove	4	1	0			
	YPD	16	0.31	0.12			
Within Population Outcross	APD	15	0.27	0.12	8.35	4	0.08
	APN	14	0.21	0.11			
	SCD	13	0	0			
	Sho.Cove	3	0	0			
	YPD	13	0.31	0.13			
Inter-population Outcross	APD	13	0.15	0.10	4.920	4	0.296
	APN	9	0.11	0.11			
	SCD	14	0.14	0.10			
	Sho.Cove	4	0	0			
	YPD	17	0.35	0.12			
Hybrid Pollination	APD	17	0.18	0.10	1.917	4	0.751
	APN	12	0.17	0.11			
	SCD	8	0.13	0.13			
	Sho.Cove	4	0	0			
	YPD	13	0.08	0.08			
Self Pollination Control	APD	18	0.94	0.06	4.311	4	0.366
	APN	14	0.79	0.11			
	SCD	12	0.92	0.08			
	Sho.Cove	5	0.6	0.25			
	YPD	20	0.85	0.08			
Outcrossing Control	APD	5	0	0	14.098	4	0.007 *
	APN	12	0.25	0.13			
	SCD	9	0.11	0.11			
	Sho.Cove	2	0	0			
	YPD	16	0.63	0.13			
Open Pollination Control	APD	18	0.89	0.08	10.178	4	0.038
	APN	18	0.78	0.10			
	SCD	16	1	0			
	Sho.Cove	3	0.33	0.33			
	YPD	20	0.85	0.08			

Table 2.12. Comparisons of mean number of buds with insect damage for treatments involving bud manipulation and no manipulation for *B. longii* and *B. fernaldii* in 2000 and 2001. N = number of buds treated, significant values for p<0.05

	Braya longii in 2000					*Braya fernaldii* in 2000					*Braya longii* in 2001				
Treatment type	**N**	**Mean damage**	**SE**	**F**	**p**	**N**	**Mean damage**	**SE**	**F**	**p**	**N**	**Mean damage**	**SE**	**F**	**p**
Manipulation	362	0.57	0.03	3.92	0.048	342	0.72	0.02	80.48	<0.001	396	0.41	0.02	0.17	0.679
No manipulation	113	0.46	0.05			106	0.26	0.04			163	0.39	0.04		

Table 2.13. Species means (mm) for measured bud characters a) for bud stage 1; b) for bud stage 2; c) for bud stage 3; d) for bud stage 4. P-values calculated from nested-ANOVAs. Type III Sum of Squares was used in analysis. Significant values for p<0.006 after Bonferroni correction for multiple comparisons. Bud stage definitions in Table 2.5.

a)

Trait	*Species*	*N*	*Mean*	*SE*	*Range*	*Source*	*F*	*P*	*Significant?*
Bud width	BL	130	1.65	0.02	1.0-2.16	Species	35.16	<0.001	Yes
	BF	57	1.43	0.03	0.94-2.51	Pop(Species)	4.32	<0.001	
Carpel length	BL	130	1.32	0.02	0.73-1.87	Species	21.44	<0.001	Yes
	BF	57	1.10	0.03	0.50-1.54	Pop(Species)	1.55	0.145	
Stamen length	BL	130	1.37	0.02	0.82-1.87	Species	34.5	<0.001	Yes
	BF	57	1.14	0.03	0.51-1.36	Pop(Species)	1.69	0.102	
Anther length	BL	130	0.66	0.008	0.46-0.90	Species	56.7	<0.001	Yes
	BF	57	0.54	0.01	0.27-0.72	Pop(Species)	2.79	0.006	
Proportion of	BL	130	0.37	0.04	0-1.0	Species	3.05	0.083	No
receptive stigmas	BF	57	0.49	0.07	0-1.0	Pop(Species)	3.23	0.002	
# pollen grains	BL	81	0	0	0-0	Species	1.09	0.308	No
on stigma	BF	43	0.28	0.28	0-12	Pop(Species)	1.91	0.074	
# germinated	BL	81	0	0	0-0	Species	-	-	-
pollen grains	BF	43	0	0	0-0	Pop(Species)	-	-	
# pollen tubes in	BL	81	0	0	0-0	Species	-	-	-
style	BF	43	0	0	0-0	Pop(Species)	-	-	
# pollen tubes in	BL	81	0	0	0-0	Species	-	-	-
ovary	BF	43	0	0	0-0	Pop(Species)	-	-	

b)

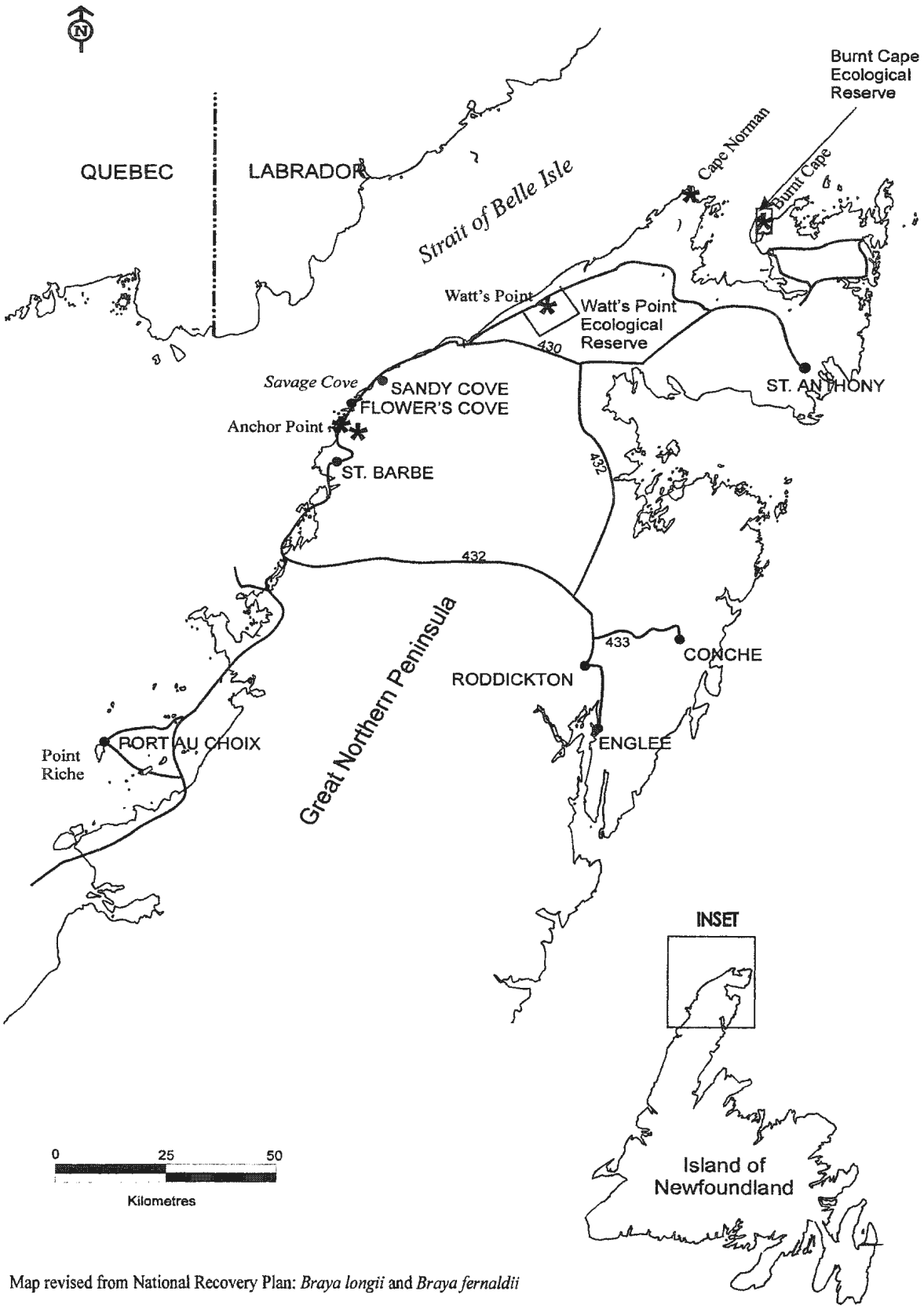
Trait	*Species*	*N*	*Mean*	*SE*	*Range*	*Source*	*F*	*P*	*Significant?*
Bud width	BL	51	1.89	0.03	1.44-2.46	Species	19.68	<0.001	Yes
	BF	59	1.66	0.03	1.25-2.31	Pop(Species)	3.05	0.004	
Carpel length	BL	51	1.56	0.02	1.15-1.74	Species	8.48	0.004	Yes
	BF	59	1.47	0.02	1.11-1.71	Pop(Species)	1.13	0.352	
Stamen length	BL	50	1.69	0.03	1.16-2.15	Species	24.09	<0.001	Yes
	BF	59	1.57	0.02	1.37-2.00	Pop(Species)	3.77	0.001	
Anther length	BL	51	0.71	0.01	0.55-0.90	Species	24.09	<0.001	Yes
	BF	59	0.65	0.01	0.50-0.79	Pop(Species)	3.77	0.01	
Proportion of	BL	51	0.78	0.06	0-1.0	Species	0	0.962	No
receptive stigmas	BF	59	0.83	0.05	0-1.0	Pop(Species)	0.01	0.053	
# pollen grains	BL	35	0	0	0-0	Species	0.066	0.432	No
on stigma	BF	59	0.25	0.13	0-5	Pop(Species)	2.16	0.039	
# germinated	BL	35	0	0	0-0	Species	0.63	0.439	No
pollen grains	BF	58	0.19	0.11	0-5	Pop(Species)	1.06	0.136	
# pollen tubes in	BL	35	0	0	0-0	Species	0.51	0.481	No
style	BF	55	0.13	0.13		Pop(Species)	0.64	0.745	
# pollen tubes in	BL	35	0	0	0-0	Species	0.51	0.481	No
ovary	BF	55	0.04	0.04	0-2	Pop(Species)	0.64	0.745	

c)

Trait	*Species*	*N*	*Mean*	*SE*	*Range*	*Source*	*F*	*P*	*Significant?*
Bud width	BL	47	2.10	0.03	1.58-2.70	Species	10.92	0.002	Yes
	BF	24	1.80	0.04	1.54-2.19	Pop(Species)	1.17	0.332	
Carpel length	BL	47	1.94	0.02	1.76-2.31	Species	3.94	0.052	No
	BF	24	1.88	0.03	1.72-2.37	Pop(Species)	1.33	0.233	
Stamen length	BL	47	1.91	0.04	1.34-2.66	Species	0.50	0.483	No
	BF	24	1.91	0.05	1.57-2.56	Pop(Species)	1.14	0.384	
Anther length	BL	47	0.77	0.01	0.63-0.91	Species	9.94	0.003	Yes
	BF	24	0.70	0.02	0.57-0.89	Pop(Species)	3.52	0.002	
Proportion of	BL	47	0.95	0.03	0-1	Species	0.34	0.565	No
receptive stigmas	BF	24	1.00	0	1-1	Pop(Species)	0.58	0.793	
# pollen grains	BL	43	0	0	0-0	Species	3.89	0.056	No
on stigma	BF	24	3.75	1.92	0-43	Pop(Species)	0.57	0.801	
# germinated	BL	43	0	0	0-0	Species	5.73	0.023	No
pollen grains	BF	21	3.19	1.66	0-33	Pop(Species)	0.92	0.509	
# pollen tubes in	BL	42	0	0	0-0	Species	6.37	0.02	No
style	BF	18	2.06	1.14	0-19	Pop(Species)	1.37	0.23	
# pollen tubes in	BL	42	0	0	0-0	Species	4.38	0.044	No
ovary	BF	17	2.29	1.57	0-26	Pop(Species)	0.75	0.647	

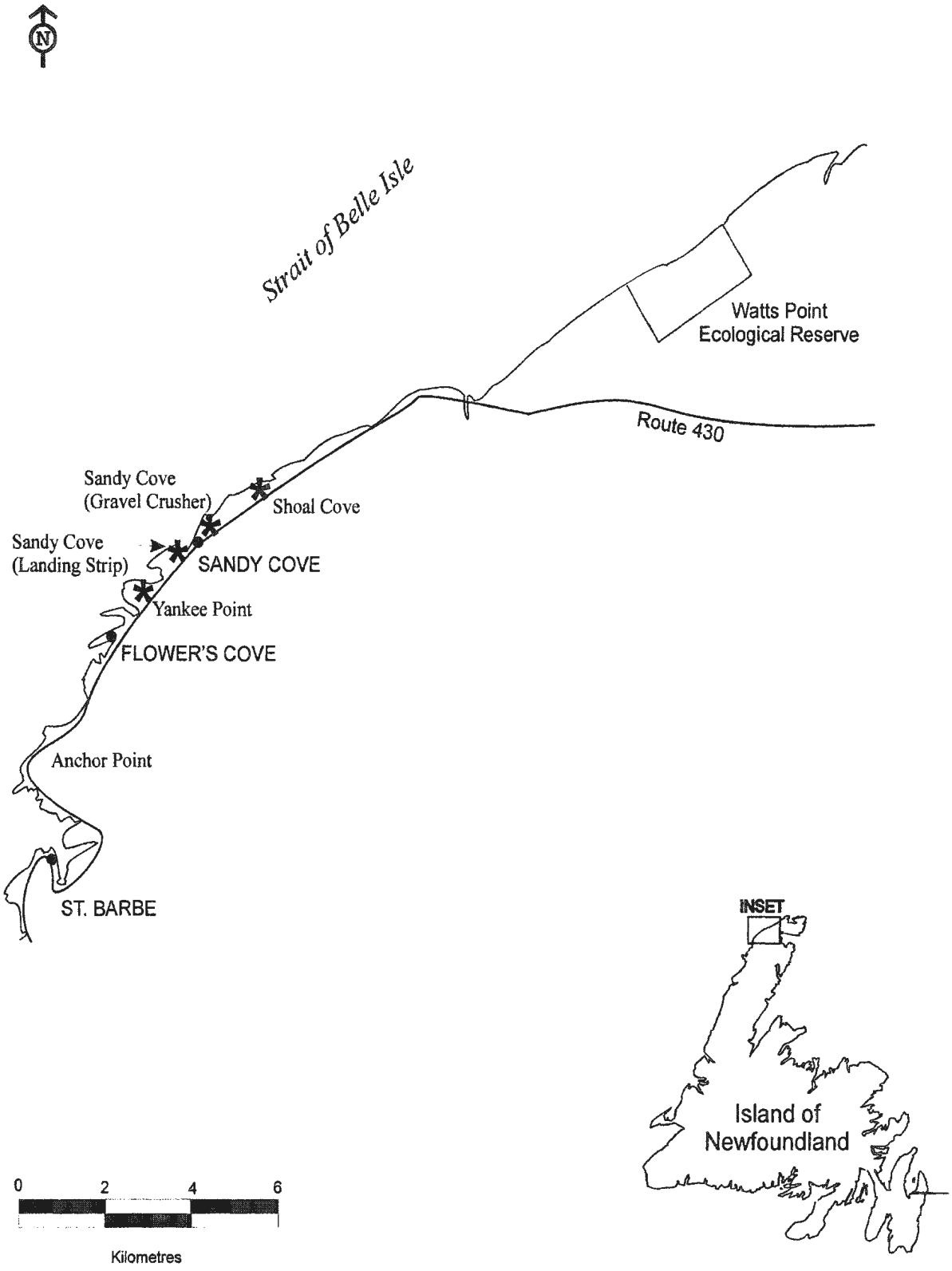
d)

Trait	*Species*	*N*	*Mean*	*SE*	*Range*	*Source*	*F*	*P*	*Significant?*
Bud width	BL	79	2.29	0.03	1.75-2.78	Species	47.05	<0.001	Yes
	BF	44	2.00	0.03	1.66-2.65	Pop(Species)	5.35	<0.001	
Carpel length	BL	78	2.01	0.03	1.45-3.05	Species	6.32	0.013	No
	BF	44	1.92	0.04	1.38-2.52	Pop(Species)	3.26	0.002	
Stamen length	BL	79	2.18	0.04	1.49-3.16	Species	1.94	0.167	No
	BF	43	2.14	0.04	1.53-2.65	Pop(Species)	2.97	0.005	
Anther length	BL	79	0.75	0.01	0.58-0.89	Species	45.57	<0.001	Yes
	BF	44	0.67	0.01	0.48-0.84	Pop(Species)	3.39	0.002	
Proportion of	BL	79	1.00	0	1-1	Species	*	*	N/A
receptive stigmas	BF	44	1.00	0	1-1	Pop(Species)	*	*	
# pollen grains	BL	69	1.13	0.60	0-31	Species	14.54	0.002	Yes
on stigma	BF	43	9.44	2.38	0-64	Pop(Species)	6.10	<0.001	
# germinated	BL	69	0.49	0.31	0-20	Species	8.23	0.012	No
pollen grains	BF	43	6.33	1.44	0-41	Pop(Species)	3.37	0.001	
# pollen tubes in	BL	69	0.23	0.19	0-13	Species	8.61	0.01	No
style	BF	43	3.77	0.99	0-23	Pop(Species)	3.16	0.002	
# pollen tubes in	BL	69	0.20	0.20	0-14	Species	1.54	0.229	No
ovary	BF	43	2.50	0.74	0-18	Pop(Species)	2.05	0.041	



Map revised from National Recovery Plan: *Braya longii* and *Braya fernaldii*

Fig. 2.1. Populations of *Braya fernaldii* used in this study. * indicates population location



Map revised from National Recovery Plan: *Braya longii* and *Braya fernaldii*

Figure 2.2. Populations of *Braya longii* used in study. * indicates population location.

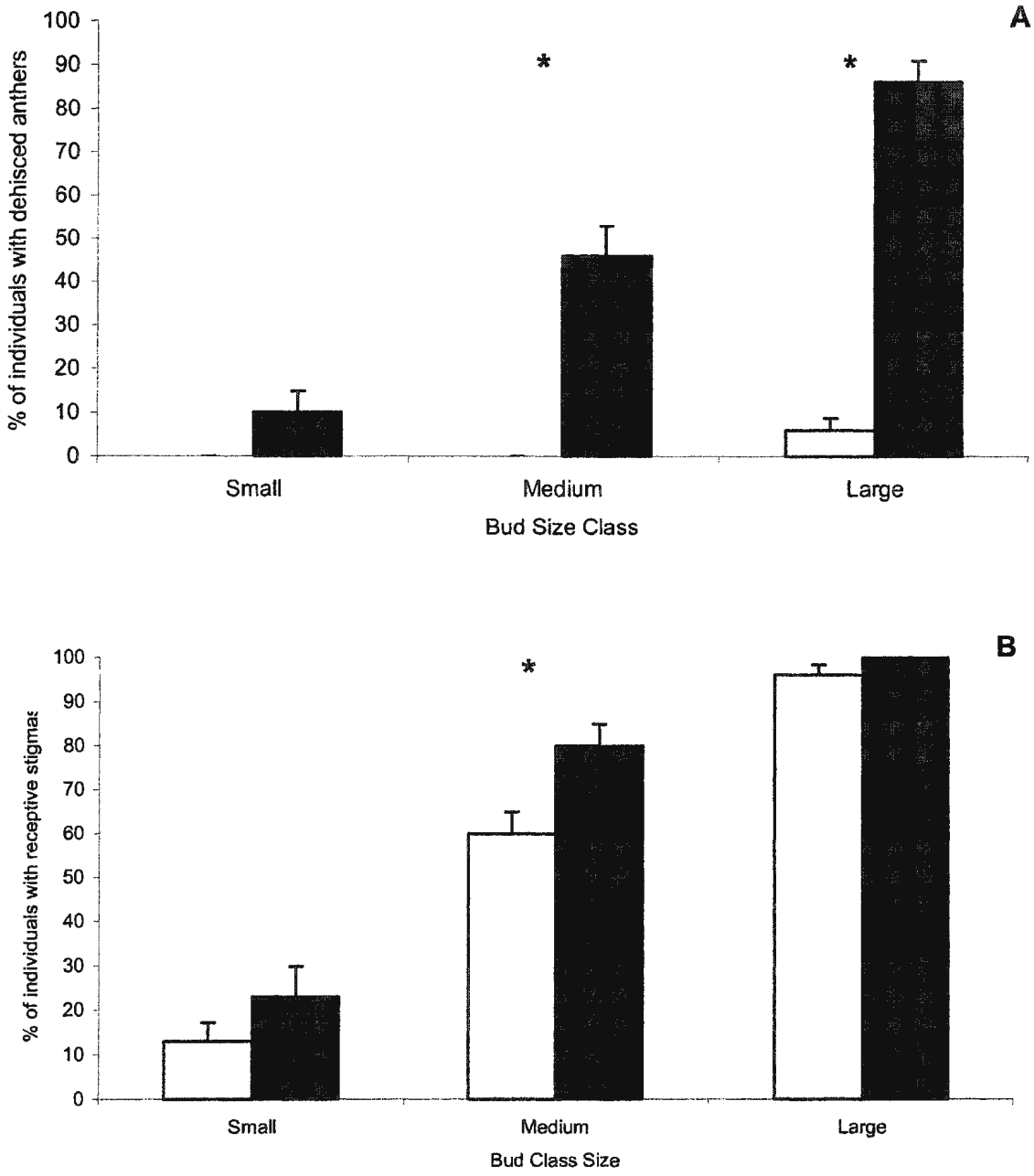


Fig. 2.3. Mean percentage of individuals a) undergoing anthesis and b) with receptive stigmas for *B. longii* (white) and *B. fernaldii* (black). * = significant difference between species ($p < 0.005$). Error bars signify standard error.

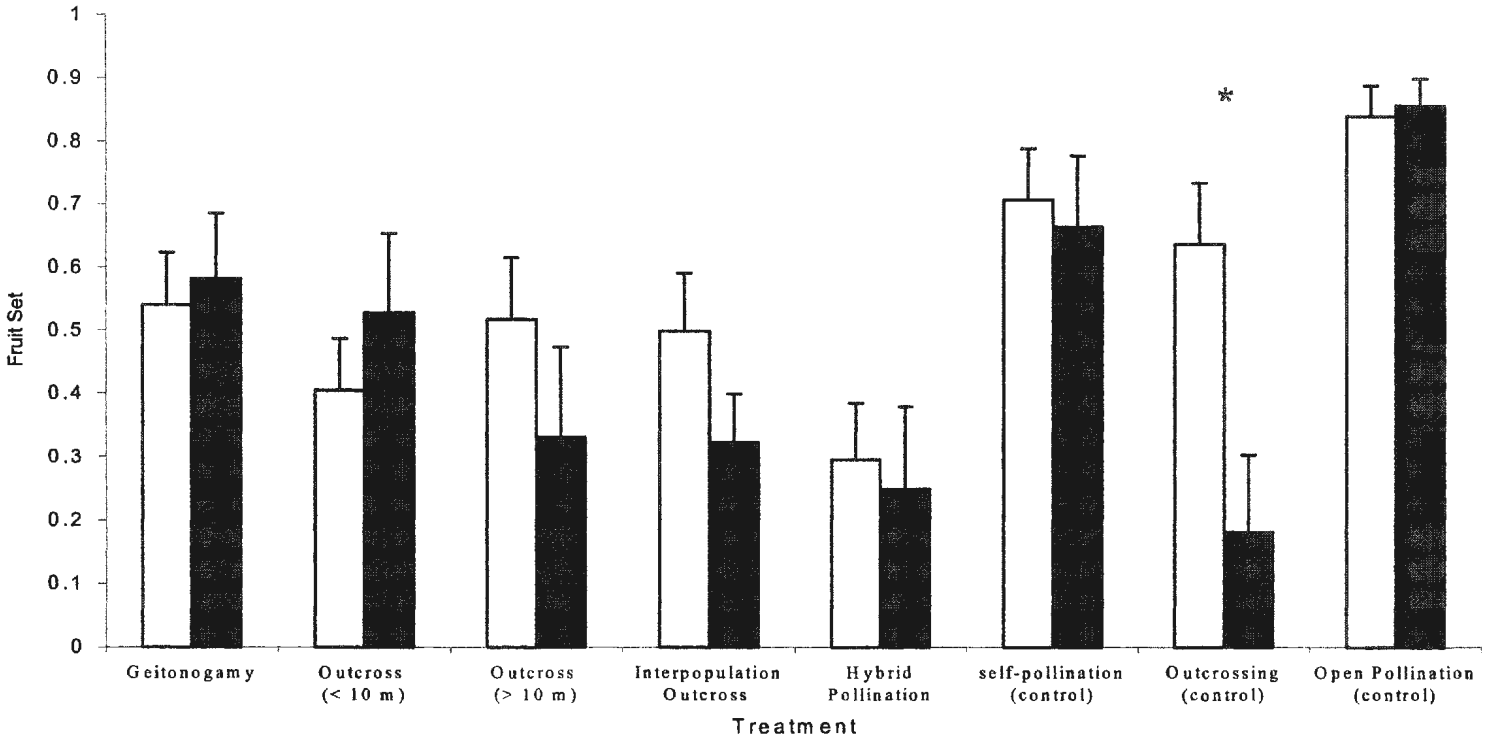


Fig. 2.4. Mean percent fruit set (# of treated buds/ # buds treated buds that produced fruit) for each hand-pollination treatment for *B. longii* (white) and *B. fernaldii* (black). * = significant difference between species (p = 0.005). Error bars signify standard error.

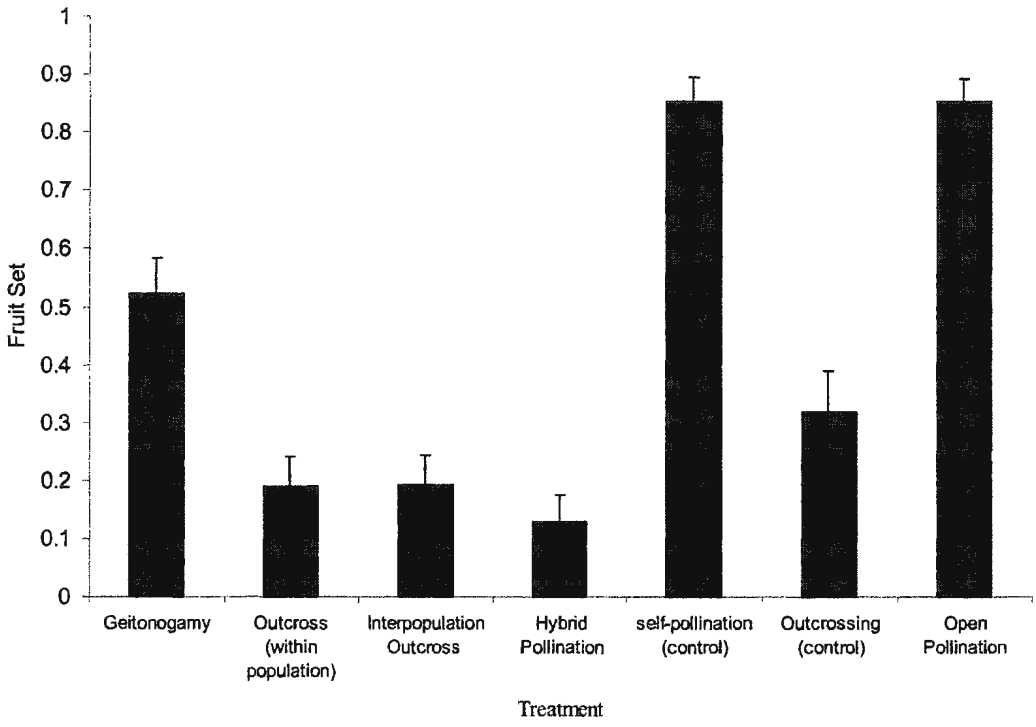


Fig. 2.5. Mean percent fruit set (# of treated buds/ # treated buds that produced fruits) for each treatment for *B. longii* in 2001. Error bars signify standard error.

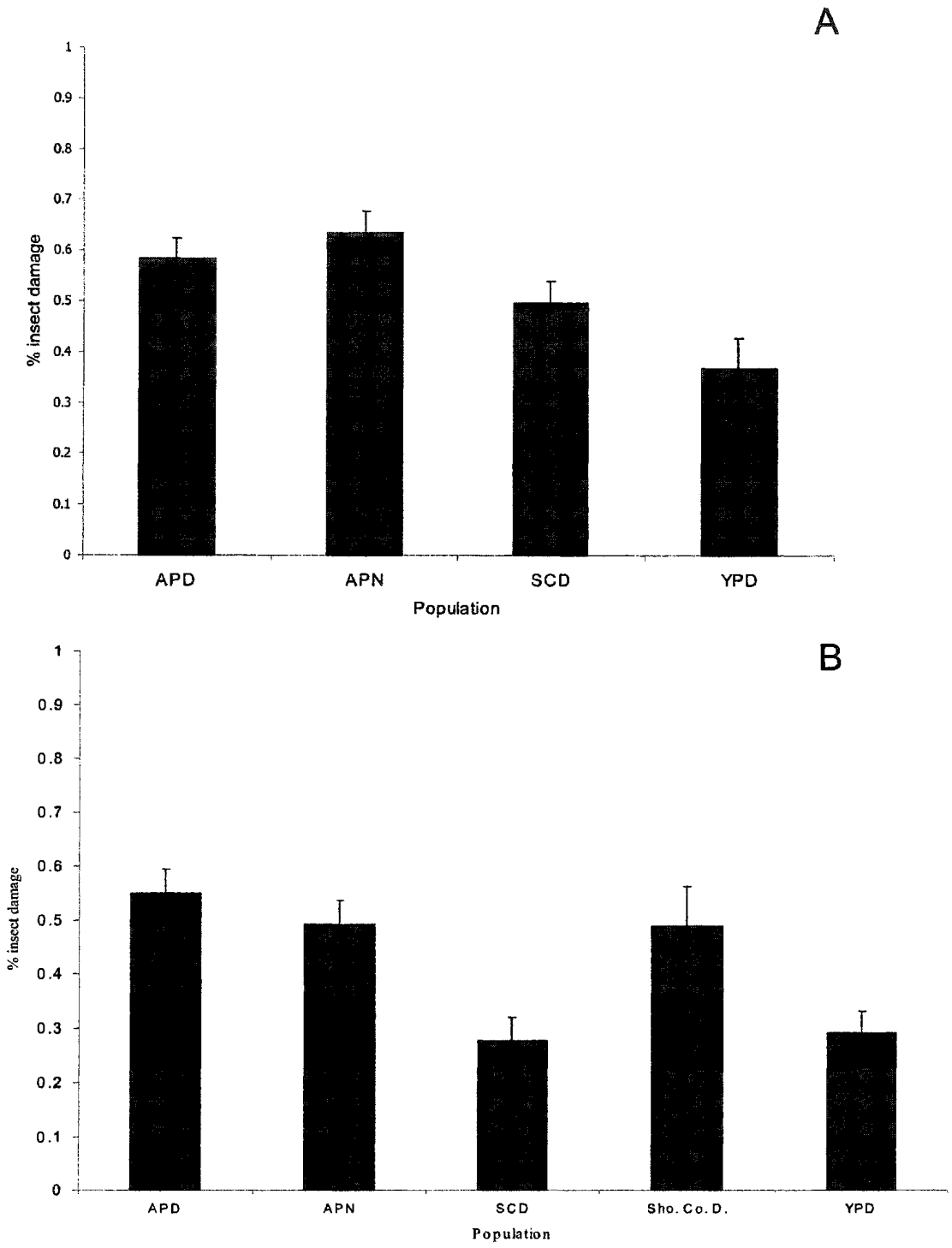


Fig. 2.6. Mean proportion of buds with insect damage among populations for *B. longii* in a) 2000 and b) 2001. Error bars signify mean standard error.

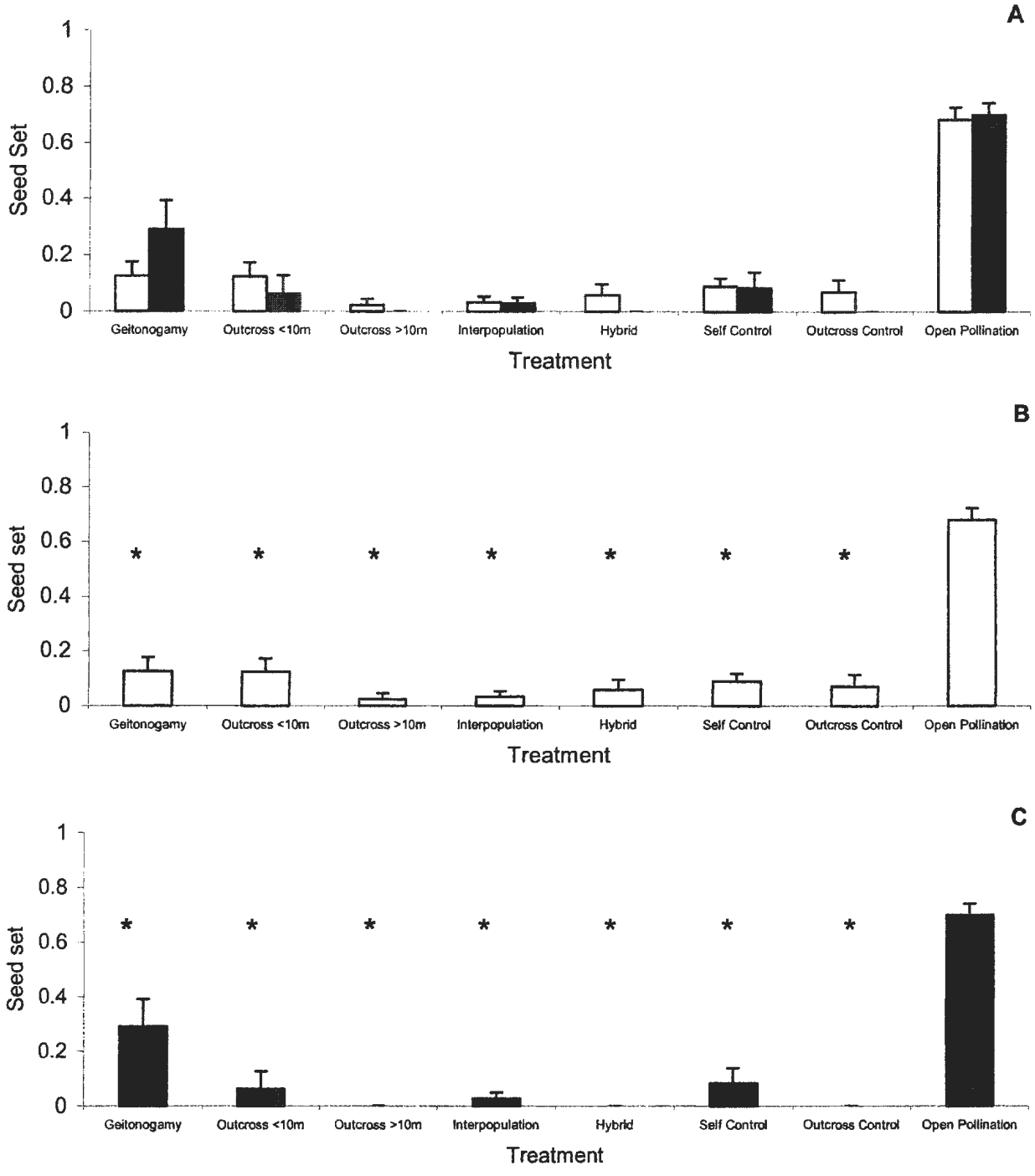


Fig. 2.7. Mean percent seed set (# seeds/ ovules) for each treatment (2000) for a) *B. longii* (white) and *B. fernaldii* (black) b) *B. longii* c) *B. fernaldii* * = significant values for seed set compared to open pollination. Empty columns = 0 seed set. Error bars signify standard error.

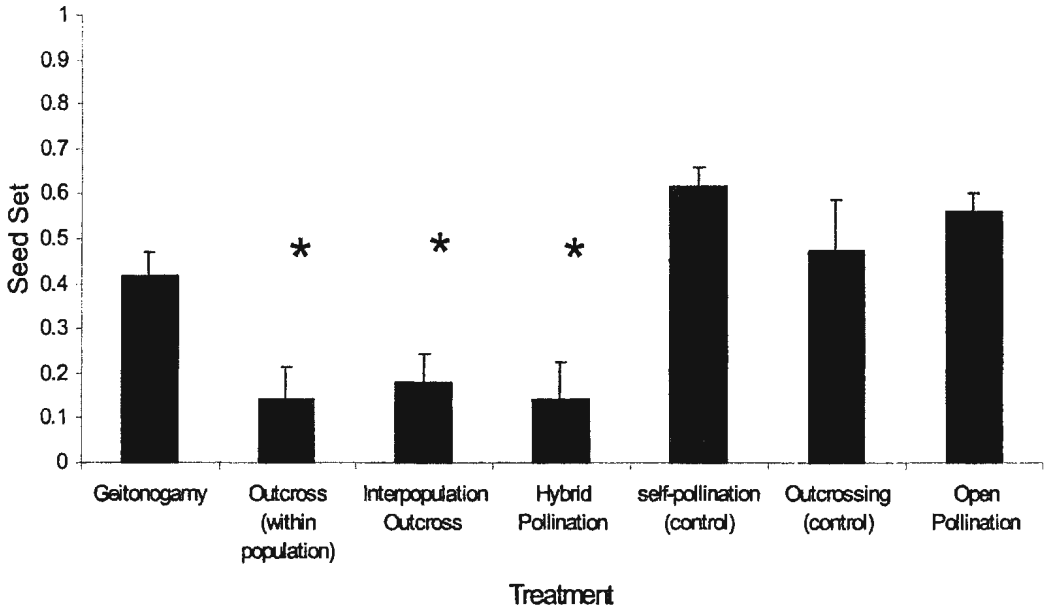


Fig. 2.8. Mean percent seed set (# seeds/ # ovules) for each hand pollination treatment for *Braya longii* (2001). * = significantly different percent seed set when compared to the control ($p < 0.008$). Error bars signify standard error.

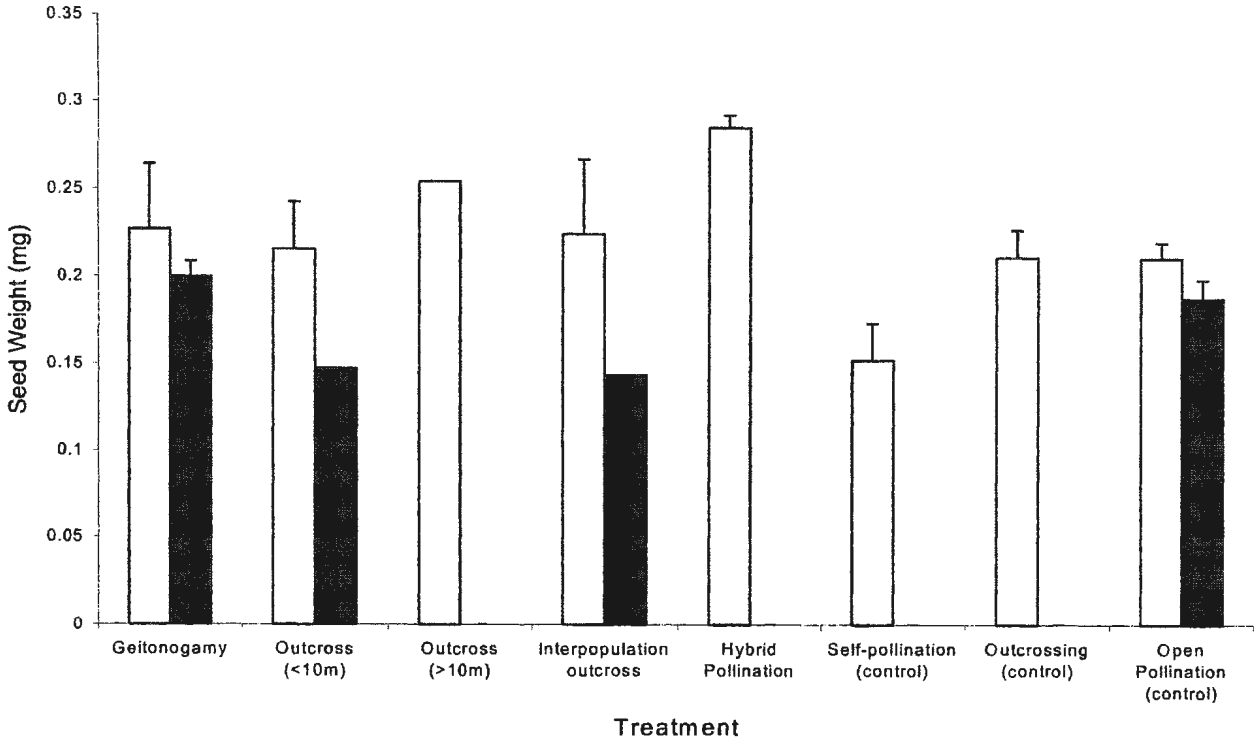


Fig 2.9. Mean seed weight (mg) for each hand-pollination treatment for *B. longii* (white) and *B. fernaldii* (black). Empty columns = no data. Error bars signify standard error.

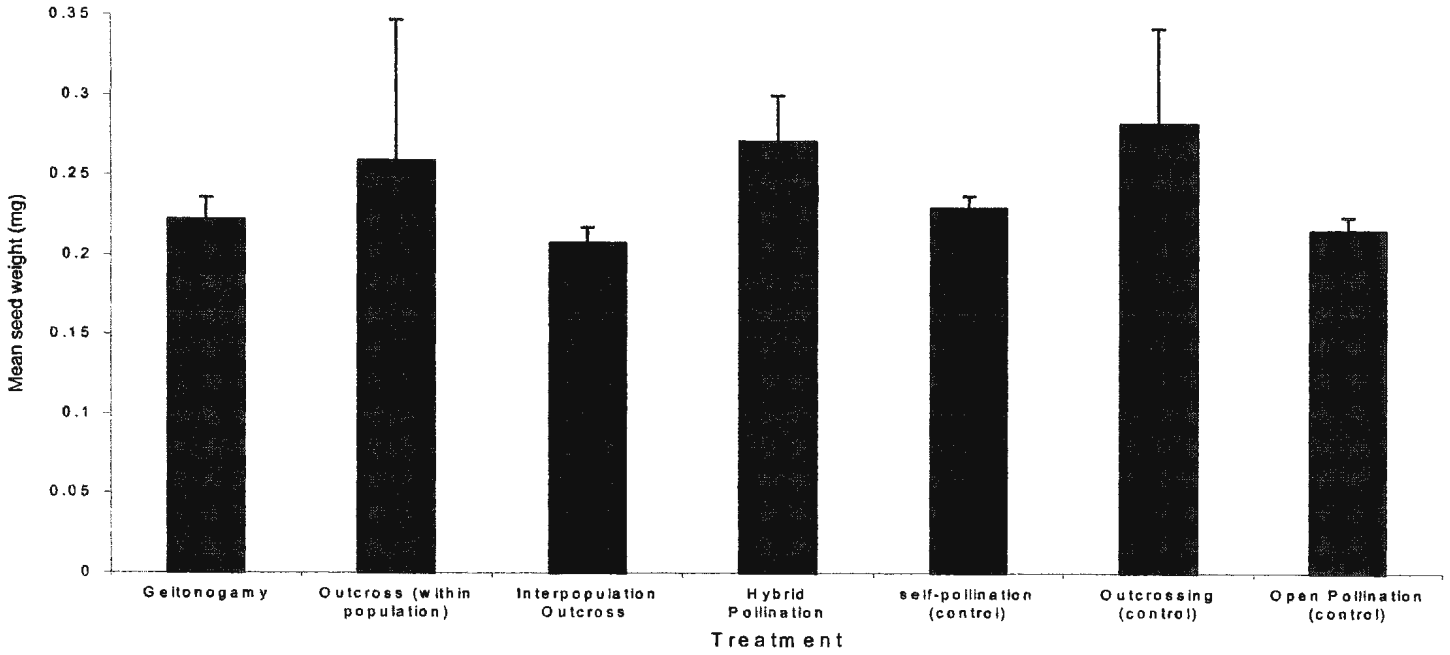


Fig 2.10. Mean seed weight (mg) for each hand-pollination treatment for *B. longii* in 2001. Error bars signify standard error.

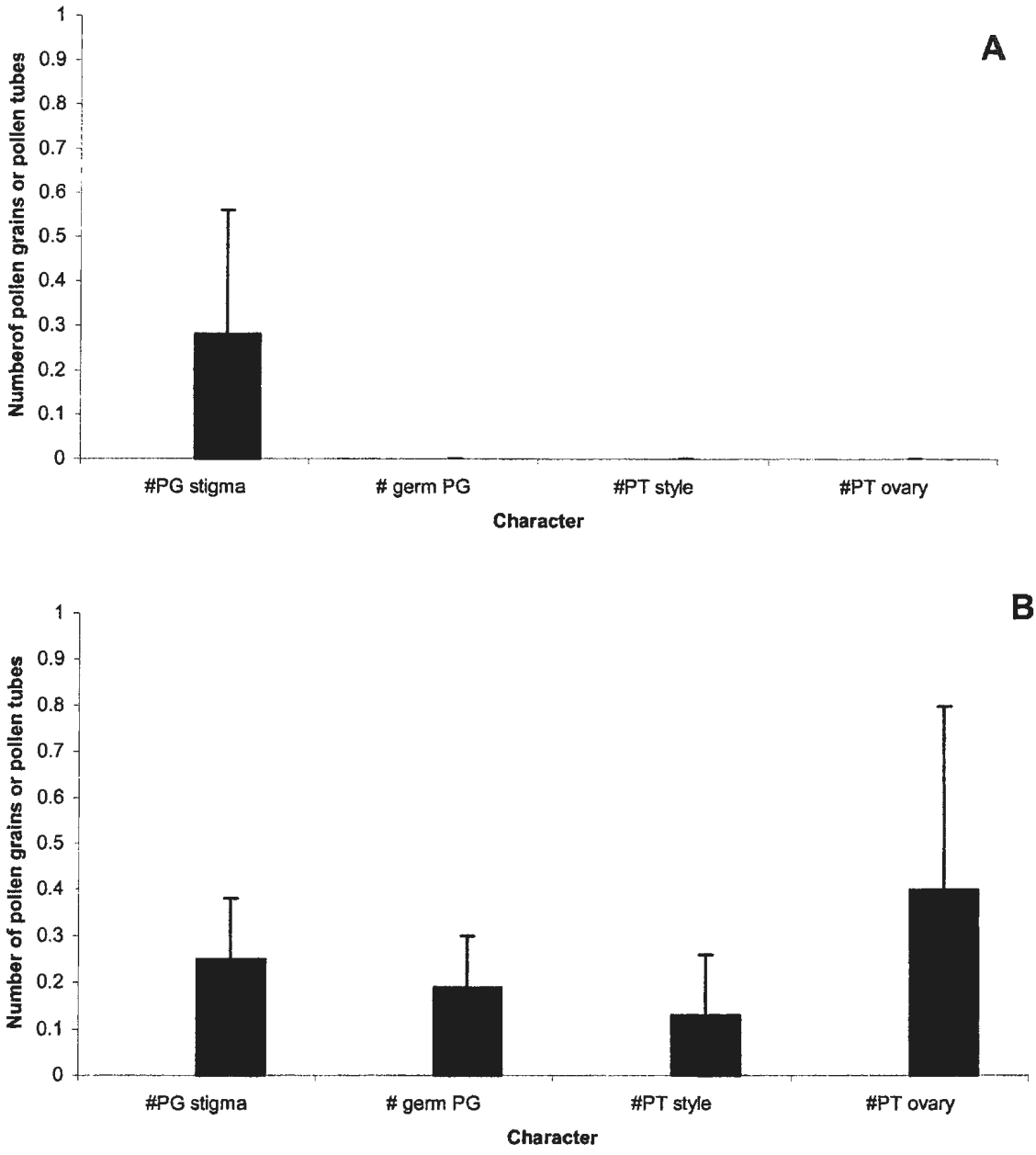
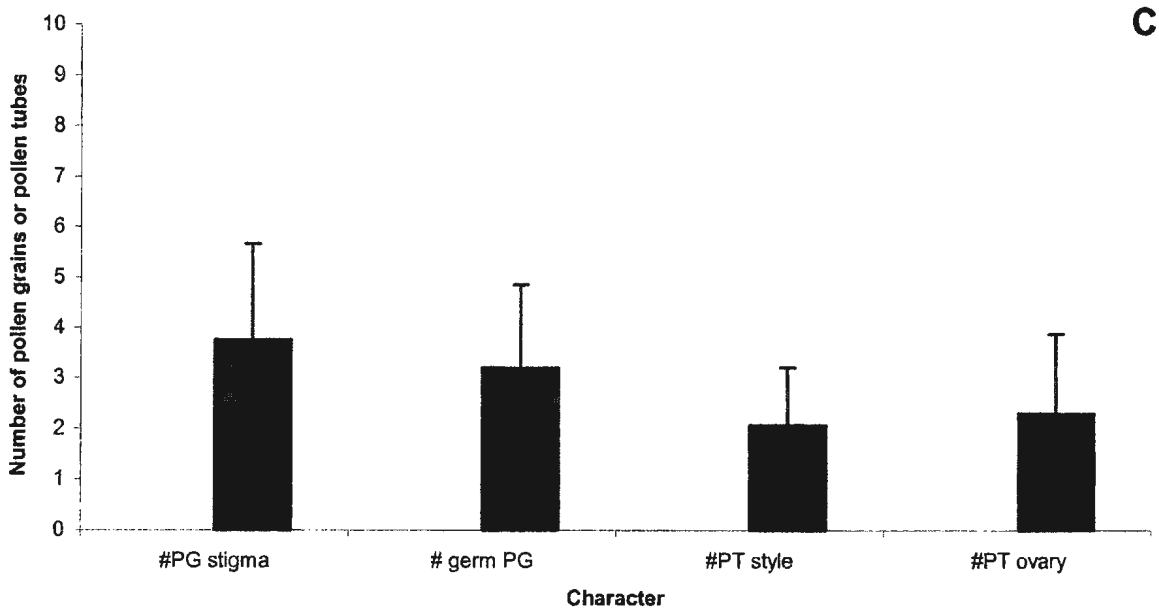
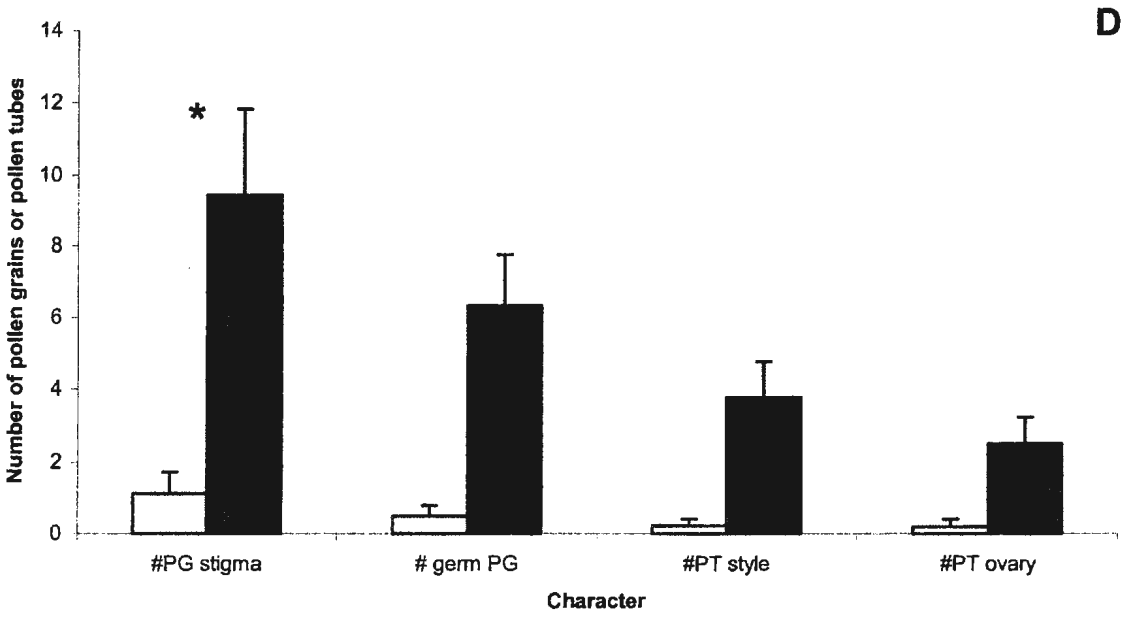


Figure 2.11. Species means for pollen tube analysis characters for a) bud stage 1; b) bud stage 2; c) bud stage 3; d) bud stage 4. * = significant values for p<0.006. PG = pollen grain; PT = pollen tube; germ = germinated. *B. longii* indicated by white, *B. fernaldii* indicated by black. Error bars signify standard error.


C
Number of pollen grains or pollen tubes
10
9
8
7
6
5
4
3
2
1
0
#PG stigma
germ PG
#PT style
#PT ovary
Character


D
Number of pollen grains or pollen tubes
14
12
10
8
6
4
2
0
*
#PG stigma
germ PG
#PT style
#PT ovary
Character

Summary and Conclusions

In this thesis I investigated the reproductive biology and floral variation in *Braya longii* and *B. fernaldii*. In Chapter 1 phenetic analysis of floral characters revealed a clear separation of species by degree of pubescence of the ovary and limb width. Future identification of the two braya species should be based on these key characters which both have a wider range than previously published. Species are not clearly differentiated based on any single character and petal length is not a good indicator of species. New species and population ranges have been established for all floral characters examined in this study. Identifying a new plant or population as either *B. longii* or *B. fernaldii* is important for conservation management, in order for risk to be assessed. Numbers of populations and numbers of plants within populations of each species must be accurately assessed to ensure viable conservation management. There was also evidence of population differentiation within both species for many of the measured characters. Therefore, in order to ensure the preservation of the total genetic variation within the two species, a representative proportion of each population must be conserved. *Ex situ* plant conservation should also represent all populations within a species to preserve this genetic variation. Protected areas must strive to encompass representatives of all populations and portions of all braya habitats must be captured in this protection.

Measured floral characters associated with plant breeding systems indicated that *B. longii*, with significantly greater numbers of pollen grains per anther, higher pollen: ovule ratios, greater degree of herkogamy as well as larger flowers, has a higher potential for outcrossing than *B. fernaldii*. Differences in the breeding biology of each species

would cause a requirement for different conservation management strategies for each species.

Individuals with intermediate floral morphology were examined using multivariate analysis to evaluate potential hybridization. Four populations contained individuals with intermediate morphology; most of them are on anthropogenically disturbed sites. The risk of hybridization becomes much greater in human-impacted sites because of movement of substrate materials, which may carry seeds to habitats beyond the species' natural dispersal range. It is possible that these intermediates are hybrids. Further studies involving molecular genetic markers would determine whether intermediate morphological characters represent hybrids in these *Braya* species as well as levels of hybridization occurring in nature. The virus infection present at Watt's Point should also be examined as well as its impact on flower and fruit formation. The presence of true hybrids may affect future recovery efforts. Increased probability of contact between the species in anthropogenically disturbed areas as the habitats come under greater development pressure should lead to careful consideration of where to do translocations or reintroductions. Only areas of natural disturbance should be used for these management tools.

In Chapter 2, potential breeding systems of *B. longii* and *B. fernaldii* were examined by hand pollinations and bud development analysis and pollen tube analysis. *Braya longii* was found to have a high potential for outcrossing while *B. fernaldii* was found to be completely autogamous with little or no potential for outcrossing. The hand pollination treatments were negatively affected by insect damage and there was low fruit

and seed set for all treatments when compared to the control. Effects of bud manipulation must be assessed in future breeding system studies of these *Braya* species. However, this may continue to be a problem since bud manipulation is necessary when performing hand pollinations in these species. Results from the hand pollination experiment supported evidence of floral characters associated with the breeding system. A primarily autogamous breeding system affects the distribution of genetic information. Autogamous plant populations usually have low genetic variation within them because the same genetic information is passed from generation to generation. This leads to problems that may increase the risk of extinction such as increased sensitivity to environmental and demographic stochasticity. The effective population size may be smaller in selfing populations, which in turn results in the requirement for a much larger minimum viable population. Therefore if a catastrophic event occurred, such as high insect damage or great anthropogenic disturbance, it is likely that all plants within the population would respond in a similar manner and the probability of extinction would increase. Thus a potential management tool would be to restore population numbers to prevent extinction. . Potential differences in the amount of outcrossing between species is also important as these differences may affect the restoration of genetic diversity within populations.

The results from the cleistogamy study supported field observations that at least one population of *B. fernaldii* contained individuals that exhibit bud pollination. Three of the four *B. fernaldii* populations examined showed evidence of cleistogamy, suggesting evolution towards fully self-fertilizing species. Cleistogamous flowers have no potential for outcrossing which again supports evidence found in the hand pollination experiment

where *B. fernaldii* have little or no potential for outcrossing. Further analysis of cleistogamy should examine buds at various positions on the flowering scape as cleistogamy can vary within an individual plant. If the earliest buds were sampled instead of late buds, the percentage of buds exhibiting cleistogamy could be much higher than what was found in this study.

Although no differences were found among populations of *B. fernaldii* with respect to the breeding system study, population differentiation was evident in *B. longii*. There was also no evidence to support inbreeding depression in either species but outbreeding depression was present in both. The hybrid pollinations resulted in some fruit production in *B. longii* but none in *B. fernaldii*, therefore, if hybridization did occur in the field, *B. longii* could act as the maternal parent and *B. fernaldii* could act as the pollen parent. This may impact future recovery efforts, especially in highly disturbed areas where contact between species is more likely to occur. Such contact has already occurred at one site (Shoal Cove). To avoid the risk of loss of both species, hybridization should be prevented. Another potential management tool would be to remove the risk by translocating plants of the invading species to an area away from the rare species. Therefore, competition would be reduced and hybridization would be less likely to compromise the persistence of the species.

Appendix 1

a) Population means for floral characters for a*) Braya longii*, b) *B. fernaldii*. Only insignificant population differences shown. See Table 1.5 a; b for descriptions of populations.

Character	*Species*	*Population*	*N*	*Mean*	*SE*	*Range*	*F*	*p*
Anther length	BL	APD	16	0.68	0.02	0.55-1.76	2.01	0.083
		APN	11	0.69	0.03	0.59-0.88		
		SCD	10	0.62	0.01	0.55-0.68		
		SCN	12	0.62	0.02	0.51-0.81		
		YPD	15	0.67	0.02	0.61-0.88		
		Sho.Co.D	15	0.65	0.01	0.57-0.74		
Anther width	BL	APD	16	0.52	0.01	0.45-0.67	3.67	0.005
		APN	11	0.56	0.02	0.45-0.67		
		SCD	10	0.51	0.01	0.44-0.58		
		SCN	12	0.52	0.02	0.44-0.61		
		YPD	15	0.52	0.02	0.42-0.67		
		Sho Co. D	15	0.58	0.02	0.48-0.68		
Stigma width	BL	APD	16	0.59	0.02	0.49-0.68	3.43	0.008
		APN	11	0.56	0.02	0.5-0.72		
		SCD	10	0.61	0.02	0.5-0.68		
		SCN	12	0.59	0.02	0.45-0.68		
		YPD	15	0.64	0.02	0.52-0.80		
		ShoCoD	15	0.56	0.01	0.44-0.64		
Abs. Herk	BL	APD	16	0.60	0.06	0.17-0.92	1.62	0.165
		APN	11	0.59	0.05	0.21-0.81		
		SCD	10	0.60	0.05	0.28-0.79		
		SCN	12	0.63	0.07	0.28-1.14		
		YPD	15	0.59	0.05	0.2-1.02		
		ShoCoD	15	0.44	0.04	0.19-0.65		
Abs. Exser.	BL	APD	16	0.26	0.04	0.05-0.56	1.16	0.335
		APN	11	0.13	0.04	0.01-0.36		
		SCD	10	0.16	0.03	0-0.32		
		SCN	12	0.29	0.04	0.03-0.37		
		YPD	15	0.18	0.04	0-0.54		
		Sho CoD	15	0.18	0.03	0-0.42		
Pubes-cence	BL	APD	16	1.02	0.02	1.0-1.33	1.89	0.106
		APN	11	1.12	0.06	1.0-1.5		
		SCD	10	1.03	0.03	1.0-1.33		
		SCN	12	1.00	0.00	1.0-1.0		
		YPD	15	1.00	0.00	1.0-1.0		
		ShoCoD	15	1.13	0.08	1.0-2.0		

b)

Character	*Species*	*Population*	*N*	*Mean*	*SE*	*Range*	*F*	*p*
Sepal width	BF	BCD	14	0.85	0.03	0.71-0.98	4.02	<0.005
		WPD	15	0.99	0.02	0.83-1.27		
		AncPt	17	0.94	0.02	0.81-1.11		
		CNN	15	0.92	0.02	0.78-1.09		
		STBN	15	0.88	0.03	0.72-0.84		
Anther length	BF	BCD	14	0.48	0.01	0.40-0.57	3.80	0.007

		WPD	15	0.56	0.02	0.42-0.66		
		AncPt	17	0.52	0.01	0.44-0.64		
		CNN	15	0.54	0.02	0.44-0.63		
		STBN	15	0.51	0.01	0.40-0.58		
Anther width	BF	BCD	14	0.42	0.01	0.36-0.51	3.51	0.011
		WPD	15	0.49	0.00	0.40-0.57		
		AncPt	17	0.46	0.01	0.34-0.57		
		CNN	15	0.46	0.01	0.37-0.51		
		STBN	15	0.47	0.02	0.40-0.59		
Carpel length	BF	BCD	14	2.15	0.03	1.93-2.35	3.14	0.019
		WPD	15	0.57	0.02	0.40-0.67		
		AncPt	17	2.10	0.05	1.75-2.52		
		CNN	15	1.99	0.05	1.62-2.21		
		STBN	15	1.97	0.06	1.62-2.41		
Stigma width	BF	BCD	14	0.56	0.01	0.5-0.61	0.09	0.984
		WPD	15	0.57	0.02	0.40-0.67		
		AncPt	17	0.56	0.01	0.49-0.65		
		CNN	15	0.56	0.02	0.42-0.64		
		STBN	15	0.57	0.02	0.46-0.71		
Ovary width	BF	BCD	14	0.89	0.02	0.80-0.98	1.77	0.144
		WPD	15	0.88	0.02	0.69-1.0		
		AncPt	17	0.88	0.02	0.66-1.09		
		CNN	15	0.84	0.02	0.68-0.97		
		STBN	15	0.83	0.02	0.70-1.01		
Abs. Herk	BF	BCD	14	0.12	0.02	0.03-0.28	2.83	0.031
		WPD	15	0.25	0.05	0.04-0.74		
		AncPt	17	0.26	0.03	0.06-0.6		
		CNN	15	0.18	0.03	0.005-0.44		
		STBN	15	0.24	0.04	0.025-0.51		
cw:lw ratio	BF	BCD	14	0.41	0.02	0.32-0.56	1.14	0.334
		WPD	15	0.41	0.01	0.29-0.48		
		AncPT	17	0.42	0.02	0.26-0.56		
		CNN	15	0.45	0.02	0.33-0.59		
		STBN	15	0.40	0.01	0.33-0.47		
Abs. Exser.	BF	BCD	14	0.26	0.04	0.01-0.56	0.82	0.518
		WPD	15	0.18	0.04	0-0.43		
		AncPt	17	0.25	0.05	0.01-0.6		
		CNN	15	0.31	0.06	0-0.76		
		STBN	15	0.26	0.06	0.01-0.69		

Appendix 2

Pollen tube analysis by population for a) bud stage 1; b) bud stage 2; c) bud stage 3; and d) bud stage 4. P-values calculated from One- Way ANOVAs. Significant values for p<0.006 after Bonferroni correction for multiple comparisons. BL = B. longii; BF = B. fernaldii; Pop = population; SE = mean standard error; PG = pollen grain; PT = pollen tube. Population abbreviations found in Tables 2.1 and 2.2. Bud stage descriptions in Table 2.5.

a)

Character	Species	Pop	N	Mean	SE	F	p	Species	Pop	N	Mean	SE	F	p
#PG on stigma	BL	APD	1	0	-	-	-	BF	Anc Pt	20	0	0	1.51	0.23
		APN	23	0	0				BCD	8	1.5	1.5		
		SCD	23	0	0				CNN	3	0	0		
		SCN	16	0	0				StB	12	0	0		
		ShoCo	18	0	0									
		YPD	0	-	-									
# germinated PG	BL	APD	1	0	-	-	-	BF	Anc Pt	20	0	0	-	-
		APN	23	0	0				BCD	8	0	0		
		SCD	23	0	0				CNN	3	0	0		
		SCN	16	0	0				StB	12	0	0		
		ShoCo	18	0	0									
		YPD	0	-	-									
# PT in style	BL	APD	1	0	-	-	-	BF	Anc Pt	20	0	0	-	-
		APN	23	0	0				BCD	8	0	0		
		SCD	23	0	0				CNN	3	0	0		
		SCN	16	0	0				StB	12	0	0		
		ShoCo	18	0	0									
		YPD	0	-	-									
# PT in ovary	BL	APD	1	0	-	-	-	BF	Anc Pt	20	0	0	-	-
		APN	23	0	0				BCD	8	0	0		
		SCD	23	0	0				CNN	3	0	0		
		SCN	16	0	0				StB	12	0	0		
		ShoCo	18	0	0									
		YPD	0	-	-									

b)

Character	Species	Pop	N	Mean	SE	F	p	Species	Pop	N	Mean	SE	F	p
#PG on stigma	BL	APD	2	0	0	-	-	BF	Anc Pt	17	0	0	3.77	0.016
		APN	1	0	-				BCD	17	0.88	0.41		
		SCD	14	0	0				CNN	15	0	0		
		SCN	12	0	0				StB	10	0	0		
		ShoCo	13	0	0									
		YPD	3	0	0									
# germinated PG	BL	APD	2	0	0	-	-	BF	Anc Pt	17	0	0	2.78	0.05
		APN	1	0	-				BCD	17	0.69	0.38		
		SCD	14	0	0				CNN	15	0	0		
		SCN	12	0	0				StB	10	0	0		
		ShoCo	13	0	0									
		YPD	3	0	0									
# PT in style	BL	APD	2	0	0	-	-	BF	Anc Pt	17	0	0	1.08	3.65
		APN	1	0	-				BCD	17	0.54	0.54		
		SCD	14	0	0				CNN	15	0	0		
		SCN	12	0	0				StB	10	0	0		
		ShoCo	13	0	0									
		YPD	3	0	0									
# PT in ovary	BL	APD	2	0	0	-	-	BF	Anc Pt	17	0	0	1.08	3.65
		APN	1	0	-				BCD	17	0.15	0.15		
		SCD	14	0	0				CNN	15	0	0		
		SCN	12	0	0				StB	10	0	0		
		ShoCo	13	0	0									
		YPD	3	0	0									

c)

Character	Species	Pop	N	Mean	SE	F	p	Species	Pop	N	Mean	SE	F	p
#PG on stigma	BL	APD	2	0	0	-	-	BF	Anc Pt	2	0.5	0.5	0.54	0.661
		APN	13	0	0				BCD	16	4.94	2.81		
		SCD	11	0	0				CNN	5	0	0		
		SCN	7	0	0				StB	1	10	-		
		ShoCo	6	0	0									
		YPD	3	0	0									

# germinated PG	BL	APD	2	0	0	-	-	BF	Anc Pt	2	0.5	0.5	0.79	0.518
		APN	13	0	0				BCD	16	4.23	2.55		
		SCD	11	0	0				CNN	5	0	0		
		SCN	7	0	0				StB	1	11	-		
		ShoCo	6	0	0									
		YPD	3	0	0									
# PT in style	BL	APD	2	0	0	-	-	BF	Anc Pt	2	0	0	1.02	0.413
		APN	13	0	0				BCD	16	2.9	0		
		SCD	11	0	0				CNN	5	0	0		
		SCN	7	0	0				StB	1	8	-		
		ShoCo	6	0	0									
		YPD	3	0	0									
# PT in ovary	BL	APD	2	0	0	-	-	BF	Anc Pt	2	0	0	0.53	0.669
		APN	13	0	0				BCD	16	3.56	0		
		SCD	11	0	0				CNN	5	0	0		
		SCN	7	0	0				StB	1	7	-		
		ShoCo	6	0	0									
		YPD	3	0	0									

d)

Character	Species	Pop	N	Mean	SE	F	p	Species	Pop	N	Mean	SE	F	p
#PG on stigma	BL	APD	6	0.17	0.17	0.75	0.592	BF	Anc Pt	11	6.27	2.72	1.61	0.195
		APN	15	2.00	1.68				BCD	15	14.6	4.21		
		SCD	14	0	0				CNN	12	3.33	3.33		
		SCN	15	0.07	0.07				StB	5	15.6	12.1		
		ShoCo	11	2.91	2.81									
		YPD	8	1.75	0.98									
# germinated PG	BL	APD	6	0	0	1.14	0.349	BF	Anc Pt	11	4.27	1.86	3.63	0.021
		APN	15	0.07	0.07				BCD	15	12	3.09		
		SCD	14	0	0				CNN	12	1.5	1.50		
		SCN	15	0.07	0.07				StB	5	5.4	3.33		
		ShoCo	11	1.82	1.82									
		YPD	8	1.5	0.97									
# PT in style	BL	APD	6	0	0	1.00	0.427	BF	Anc Pt	11	1.37	0.67	2.93	0.045
		APN	15	0	0				BCD	15	7.40	2.15		

		SCD	14	0	0				CNN	12	1.50	1.50		
		SCN	15	0	0				StB	5	3.60	2.40		
		ShoCo	11	1.18	1.18									
		YPD	8	0.4	0.4									
# PT in ovary	BL	APD	6	0	0	1.06	0.40	BF	Anc Pt	11	1.09	0.9	2.66	0.062
		APN	15	0	0				BCD	15	5.13	1.45		
		SCD	14	0	0				CNN	12	1.5	1.5		
		SCN	15	0	0				StB	5	0	0		
		ShoCo	11	1.27	1.27									
		YPD	8	0	0									